Aplicaciones de la citometría de flujo en la práctica clínica

Diagnóstico de síndromes linfoproliferativos

Bioqca. María Belén Venegas

Los **síndromes linfoproliferativos crónicos (SLPC)** constituyen un grupo heterogéneo de enfermedades caracterizadas por la **expansión clonal** de células linfoides de aspecto maduro que reflejan las características de distintas subpoblaciones normales de **linfocitos maduros.**
Desde el punto de vista fenotípico y de acuerdo con su origen, los SLPC se clasifican en SLPC-B, T y NK

APORTES de la CMF en el estudio de SLPC-B

- **DIAGNÓSTICO**:

  Diferenciar NORMAL-REACTIVO-NEOPLÁSICO

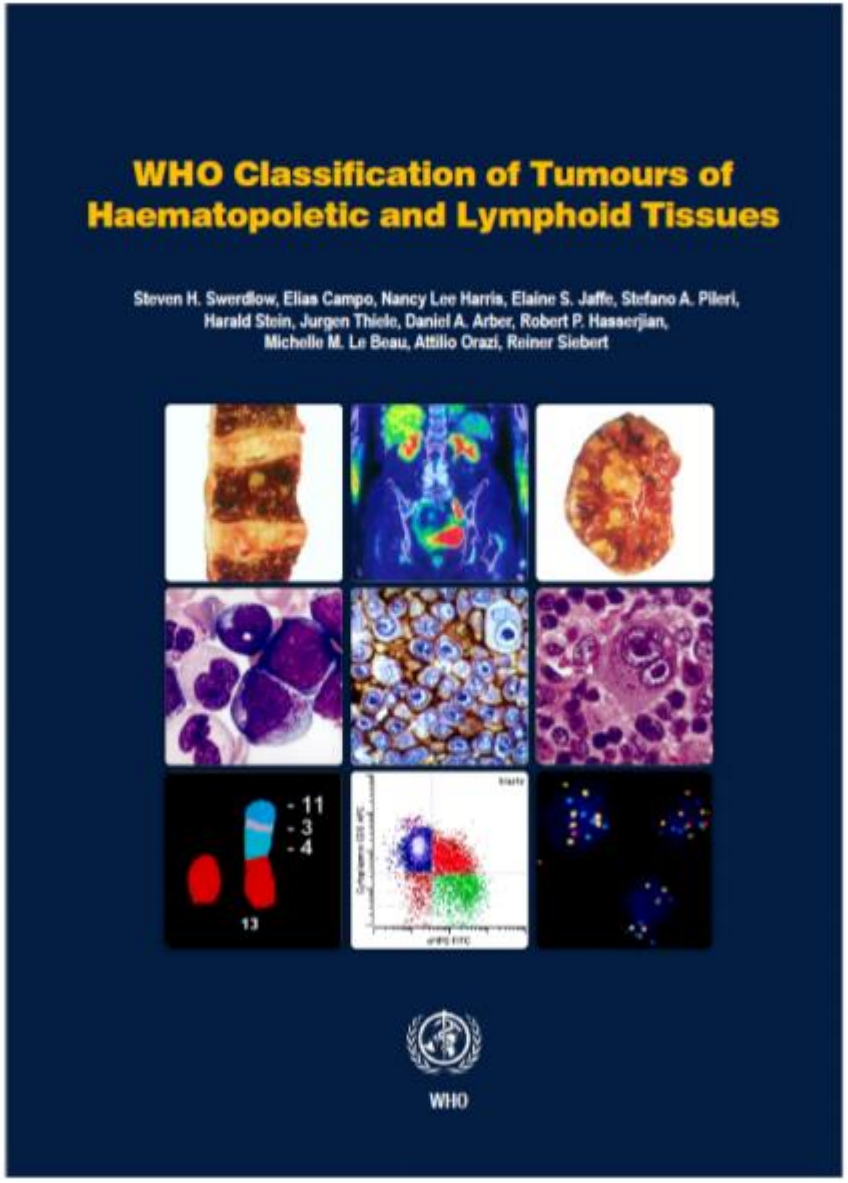
- **CLASIFICACIÓN**: (OMS 2016)

  - Estadificación, pronóstico.

  - Reconocimientos de grupos de riesgo diferente, nuevos protocolos de tratamiento

- **TRATAMIENTO**: Evaluación de EFECTIVIDAD del Tratamiento, detección de EMR

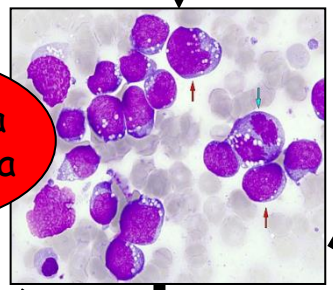
OMS 2016


WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues
Steven H. Swerdlow, Elias Campo, Nancy Lee Harris, Elaine S. Jaffe, Stefano A. Pileri, Harald Stein, Jurgen Thiele, Daniel A. Arber, Robert P. Hasserjian, Michelle M. Le Beau, Attilio Orazi, Reiner Siebert
- 11
- 3
- 4
13
WHO

Diagnóstico de Neoplasias hematológicas

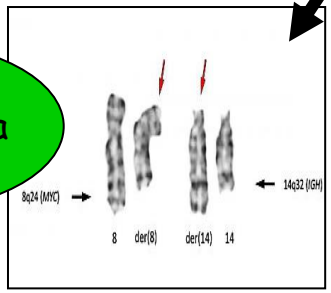
Clínica:
Signos y Síntomas

Hallazgos de laboratorio

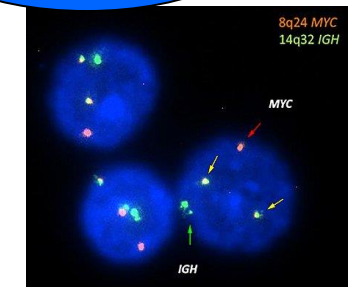


Morfología Citoquímica

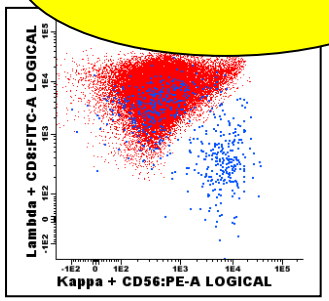
Citogenética

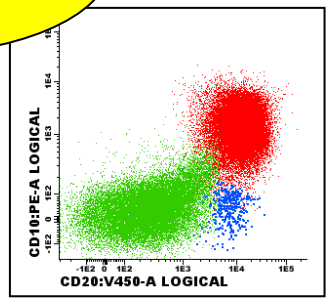


Biología Molecular FISH



INMUNOFENOTIPO





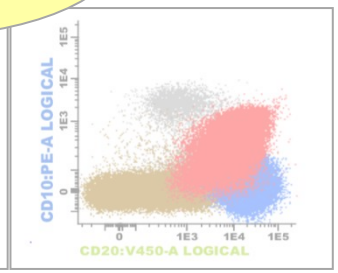
Diagnóstico de Neoplasias hematológicas

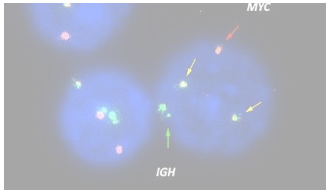
Clínica:
Signos y S

Hallazgos de
oratorio

Citogenética

FENOTIPO

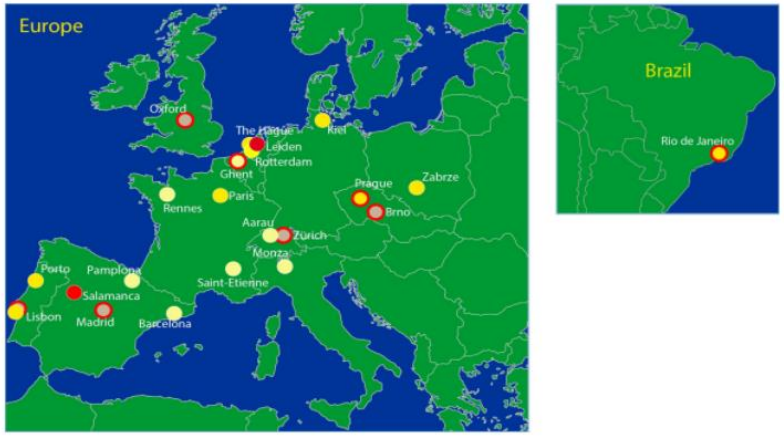




EuroFlow

It is an independent scientific consortium that aims at innovation and standardization of flow cytometric immunophenotyping to further improve and progress diagnostic patient care.

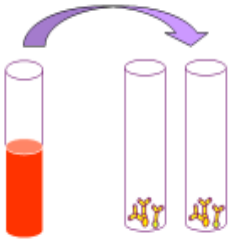
EuroFlow Consortium Participants



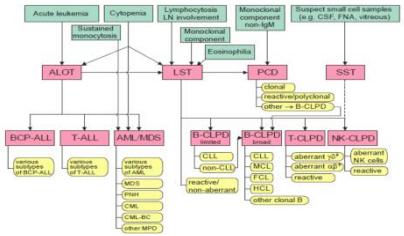
JJM Van Dongen and A Orfao . Leukemia (2012) 26, 1899-1907

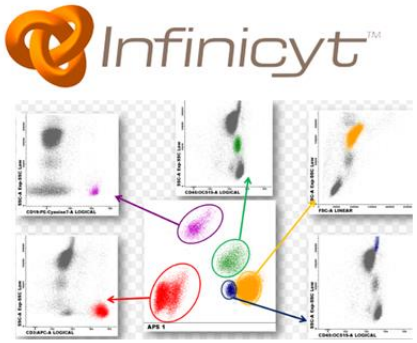
EuroFlow

- Uso de 3 láseres: 8 colores
- Estandarización del setting del instrumento (beads de estandarización-rainbow beads)
- Estandarización del protocolo de procesamiento de la muestra (SOPs),



- Selección optima de flurocromos y de los clones de AcMo.
- Diseñar combinaciones de tubos de 8 colores.
- Nuevos paneles y algoritmo diagnóstico .





- Nuevo software (Infinicyt) para el análisis de datos multivariables de los patrones inmunofenotipicos de las células normales y aberrantes., comparación de archivos de referencia.

TIPOS DE MUESTRAS

- Sangre periférica
- Médula ósea

} Con EDTA o Heparina
Temp. amb

- Ganglio, bazo
- Tejidos sólidos
- Piel
- Biopsias de mucosas

} Con sol. fisiol/ RPMI
4°C

- Líquidos de punción: LCR, líquido pleural → Con heparina/TransFix

Células en suspensión

EuroFlow

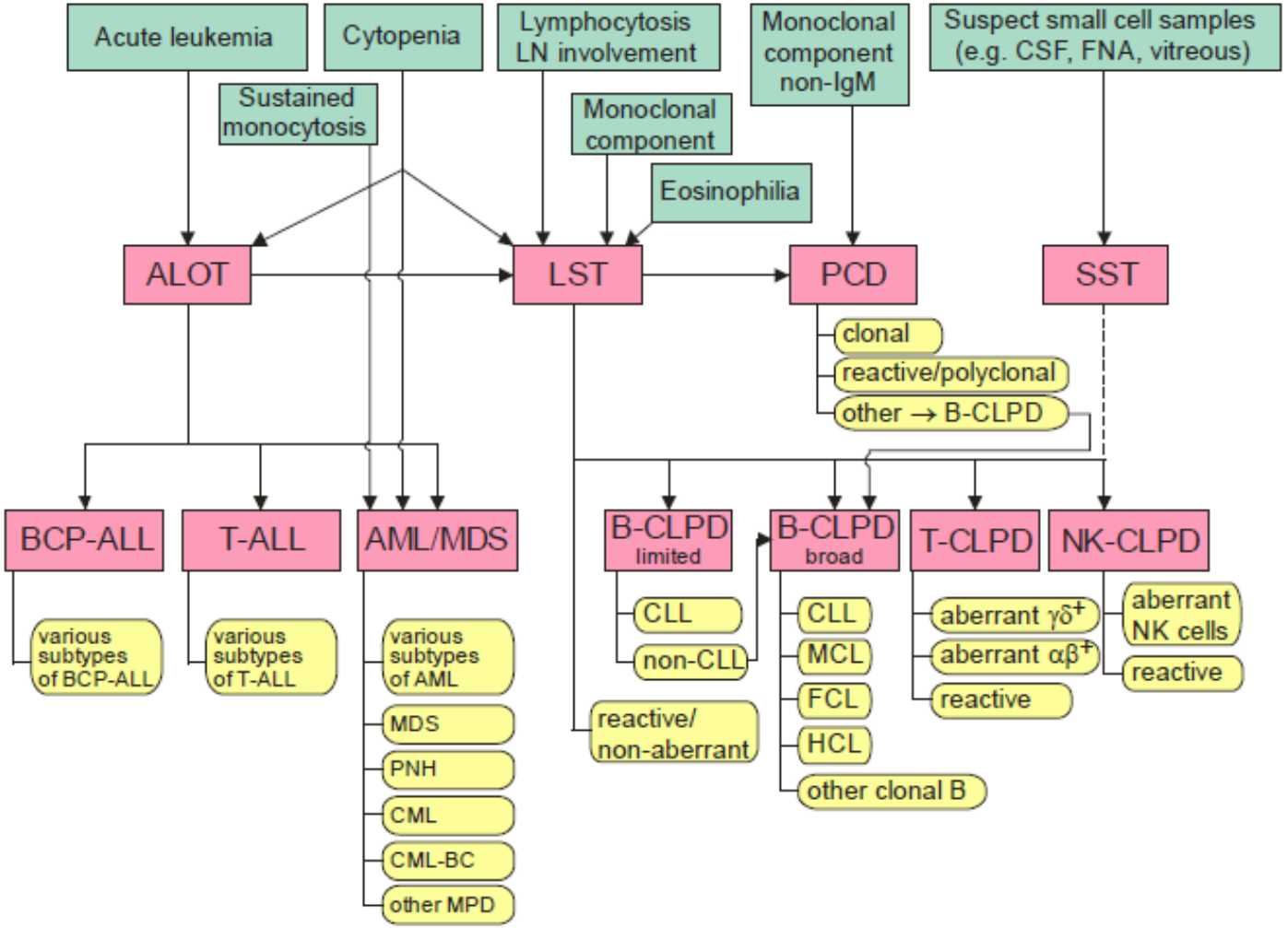
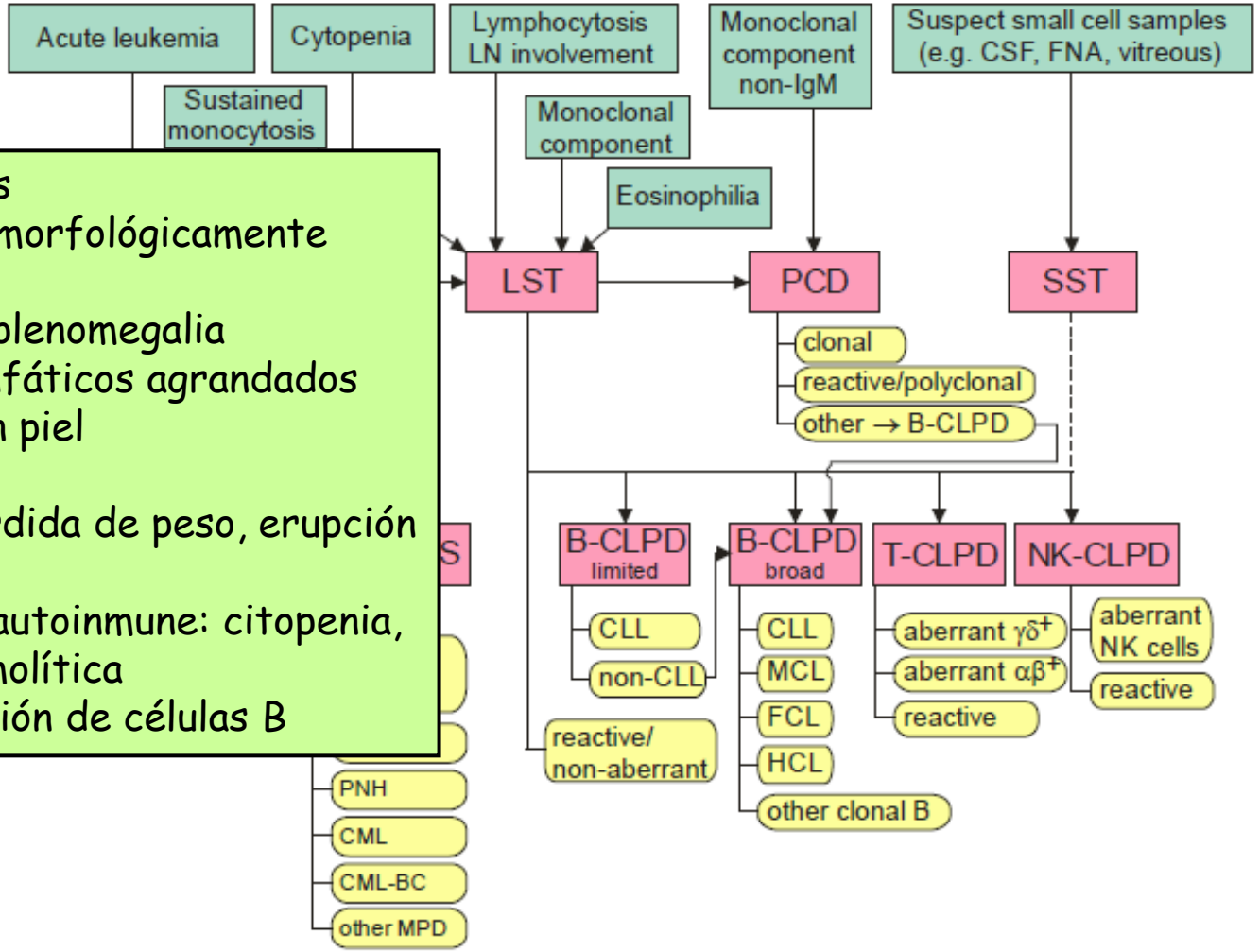


Figure 1. Diagnostic flow diagram showing the potential usage of the EuroFlow antibody panels. Multiple entries are possible, which are dependent on the clinical-diagnostic question and on the availability of cell material. For example, analysis with the "small sample tube" (SST) can be followed by analysis with additional antibody panels (see dotted line), if sufficient cell material is left over.

Van Dongen et al, Leukemia 2012;26:1908-1975

EuroFlow

Linfocitosis
Linfocitos morfológicamente anormales
Hepato/esplenomegalia
Ganglios linfáticos agrandados
Lesiones en piel
Eosinofilia
Fiebre, pérdida de peso, erupción dérmica
Fenómeno autoinmune: citopenia, anemia hemolítica
Desregulación de células B



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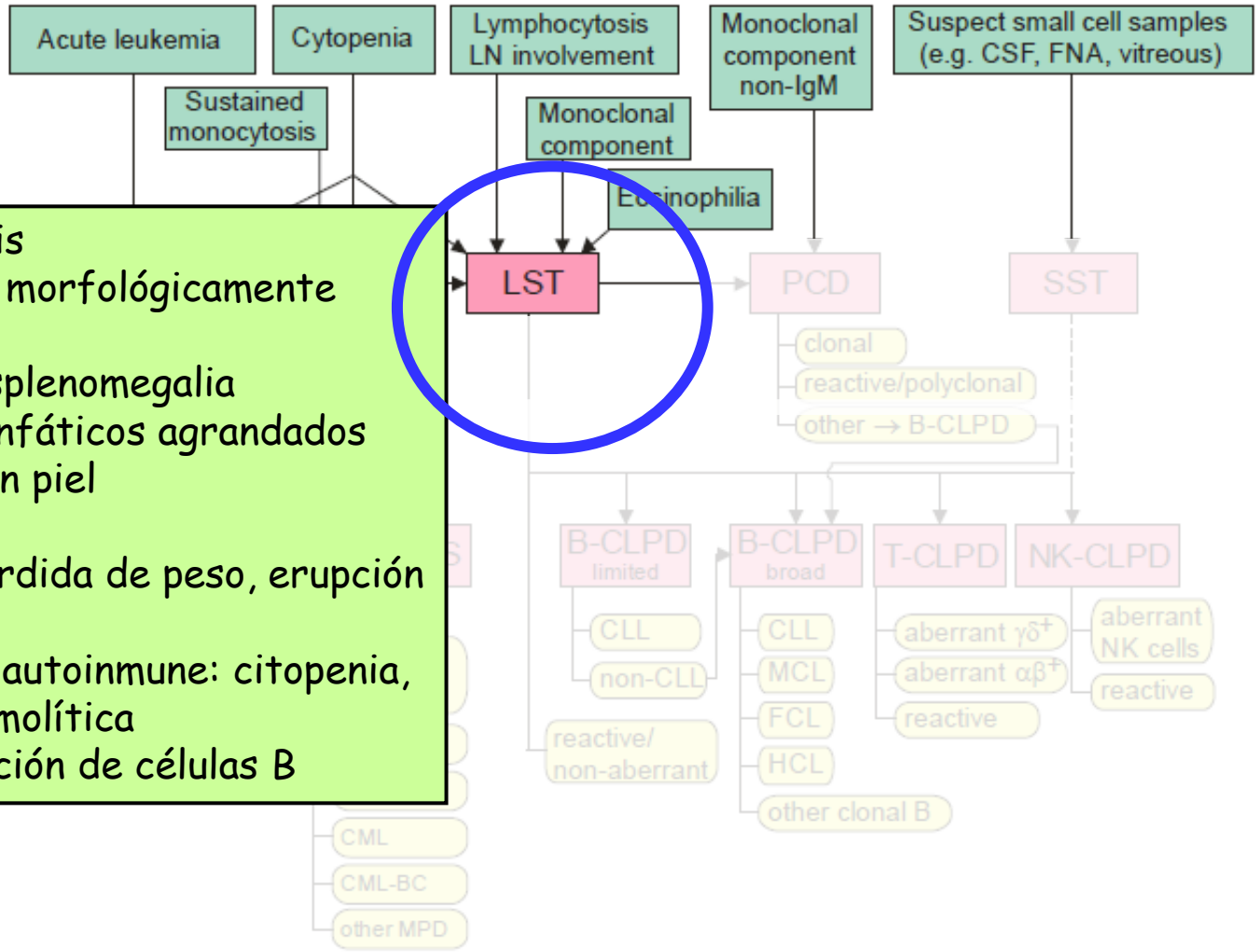

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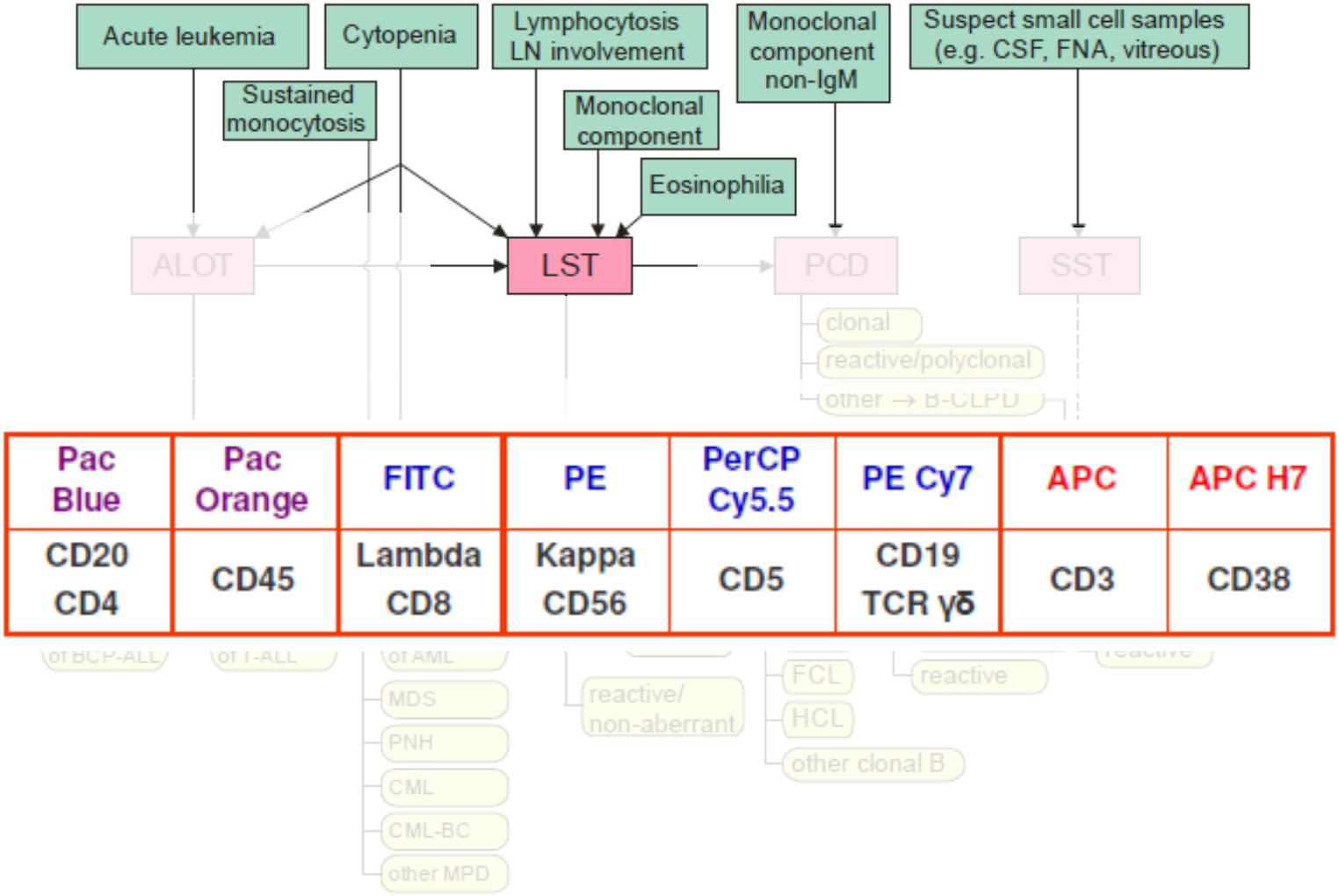
EuroFlow



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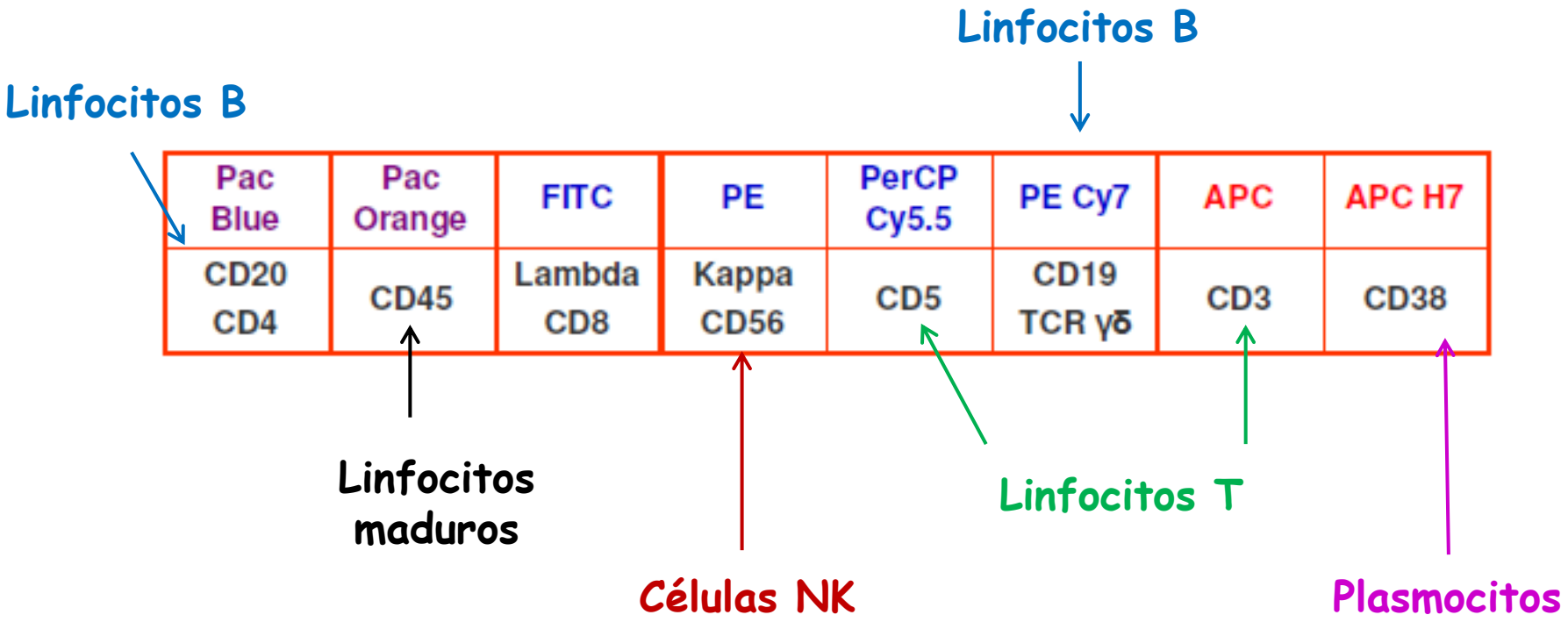
EuroFlow

LST(Tubo de Screening Linfoide)



*Identificar linaje B: CD20, CD19 y CD38. Aberraciones B más comunes: CD5, y estudiar clonalidad: Lambda y Kappa.
*Identificar células T y los sub-sets de células T: CD3, CD4 y CD8. TCRgd. Aberraciones T más frecuente: CD5.
*Marcadores para identificar las células NK: CD56. Y subtipos: CD8.

LST(Tubo de Screening Linfoide)

Linfocitos B

Linfocitos B

Pac Blue	Pac Orange	FITC	PE	PerCP Cy5.5	PE Cy7	APC	APC H7
CD20 CD4	CD45	Lambda CD8	Kappa CD56	CD5	CD19 TCR γδ	CD3	CD38

Linfocitos maduros

Células NK

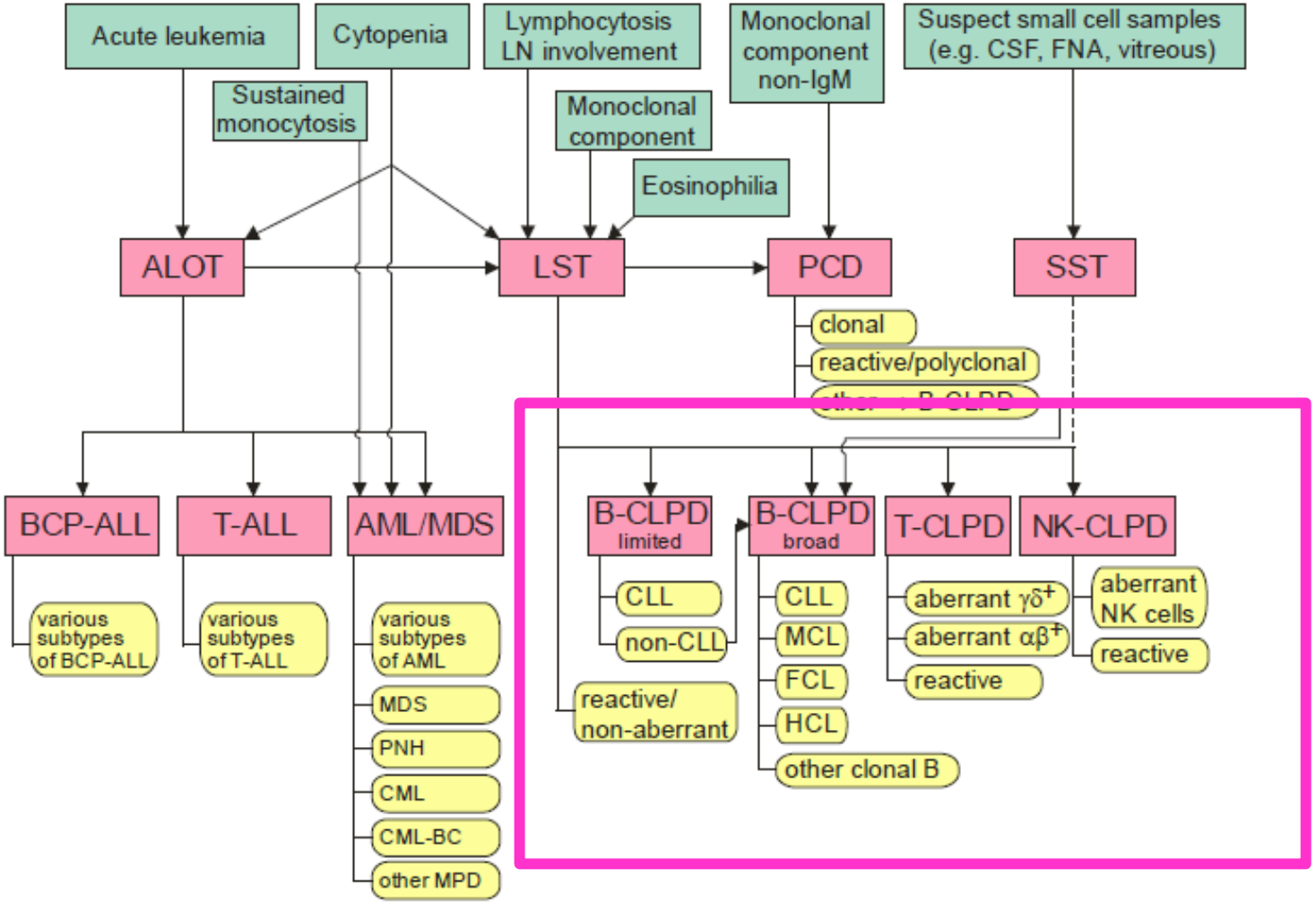
Linfocitos T

Plasmocitos

*Identificar linaje B: CD20, CD19 y CD38. Aberraciones B m
estudiar clonalidad: Lambda y Kappa.
*Identificar células T y los sub-sets de células T: CD3, CD4
Aberraciones T más frecuente: CD5.
*Marcadores para identificar las células NK: CD56. Y subtipos: CD

SLPC-B/T/ Nk

EuroFlow



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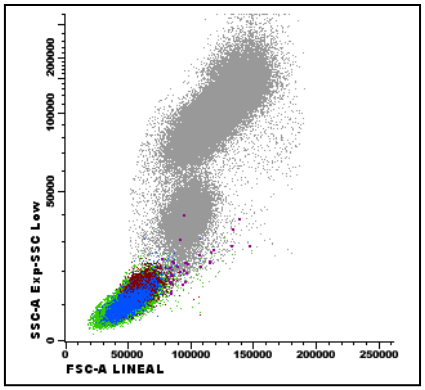
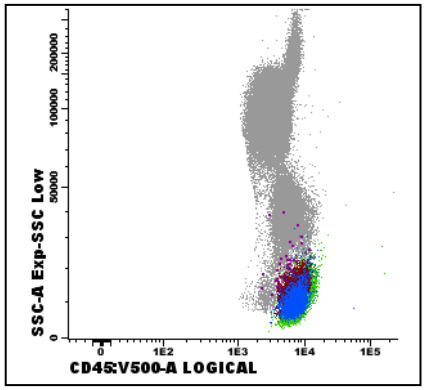
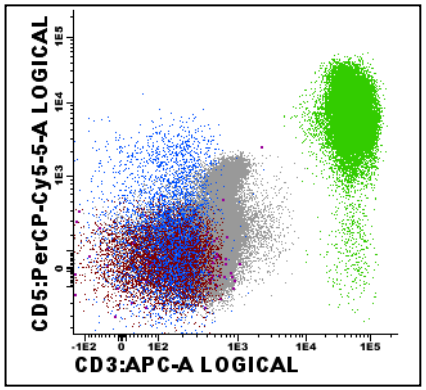
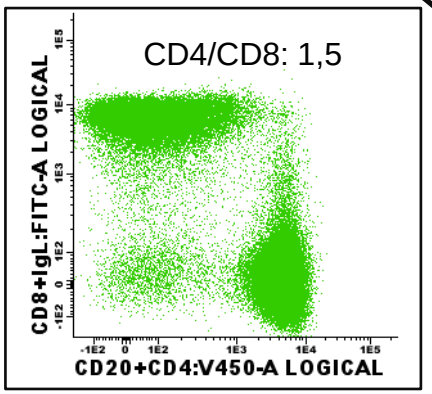
Van Dongen et al, Leukemia 2012;26:1908-1975

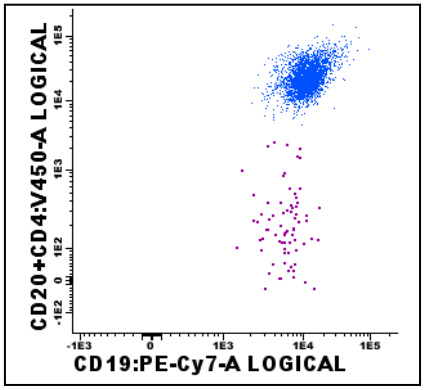
Muestra: SANGRE PERIFÉRICA

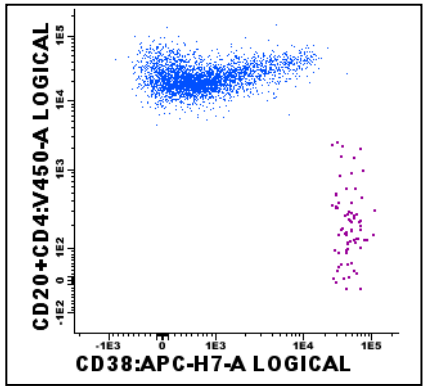


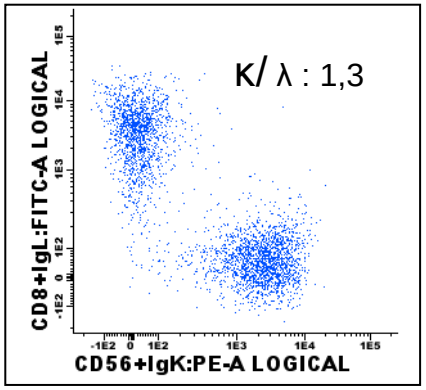


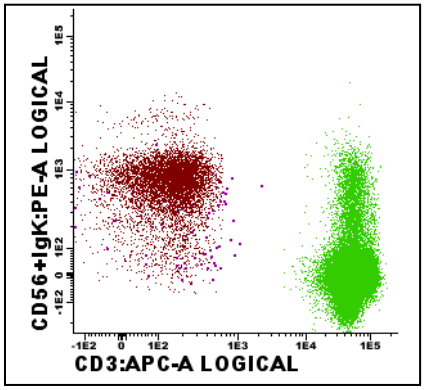






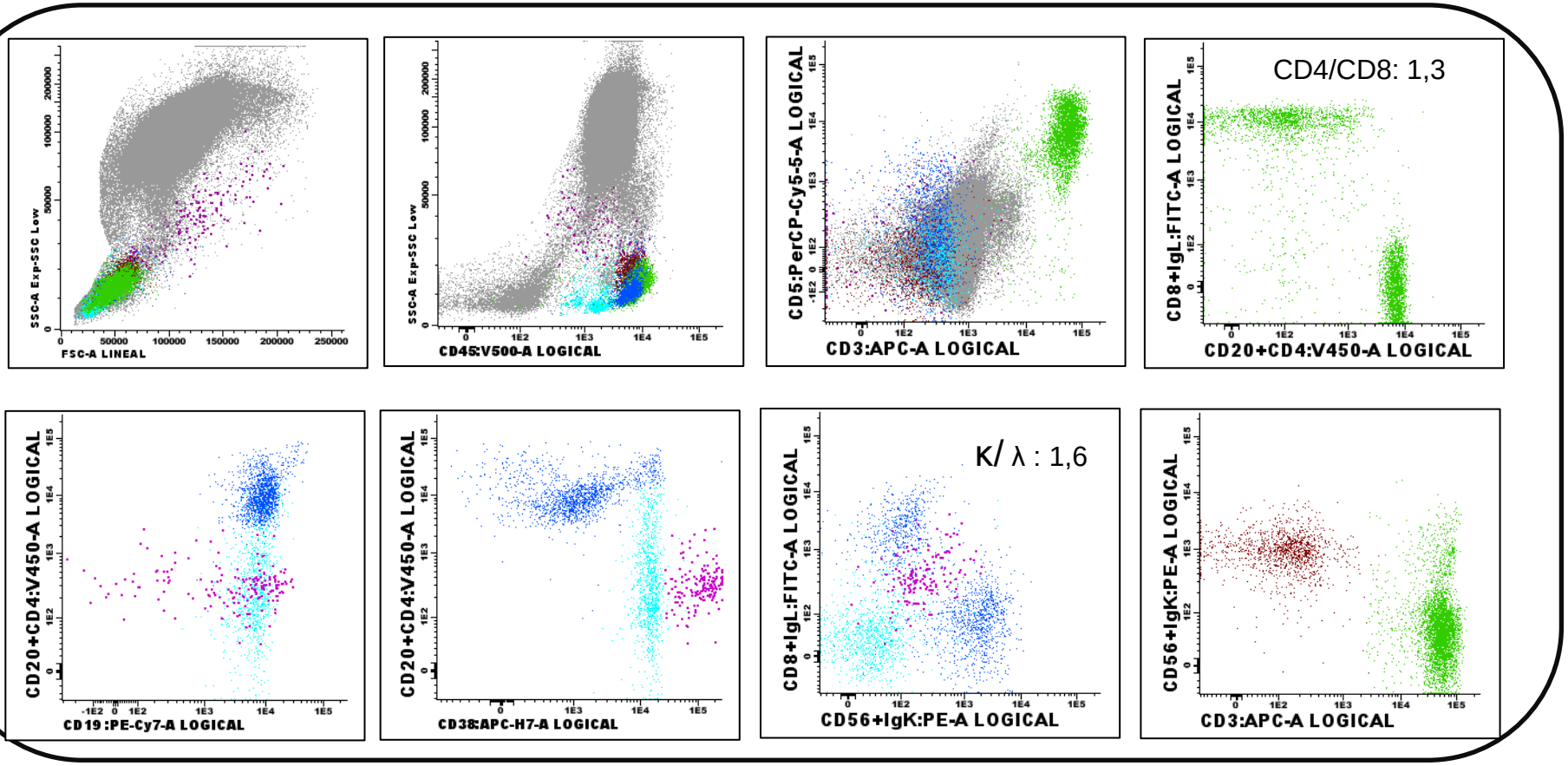






Linf T – Linf B policlonales – Cel. NK
Cel.Plasmáticas

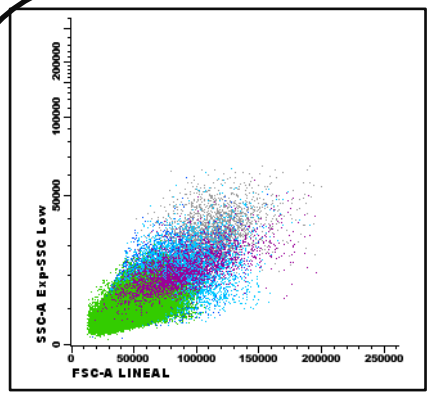
Muestra: MÉDULA ÓSEA

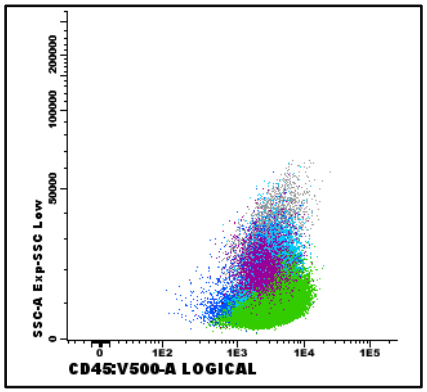


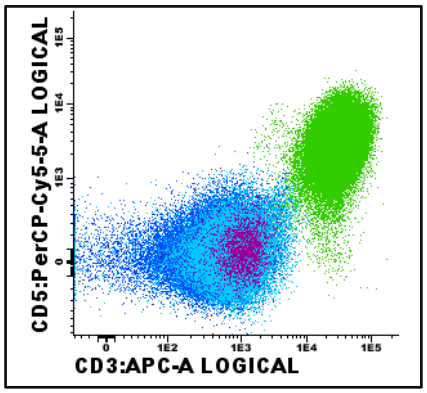
Linf T – Linf B policlonales – Linf B precursores

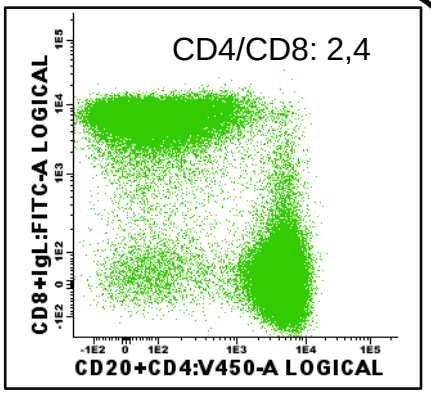
Células NK – Cel.Plasmáticas

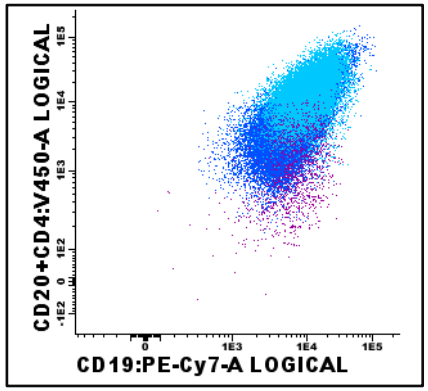
Muestra: GANGLIO

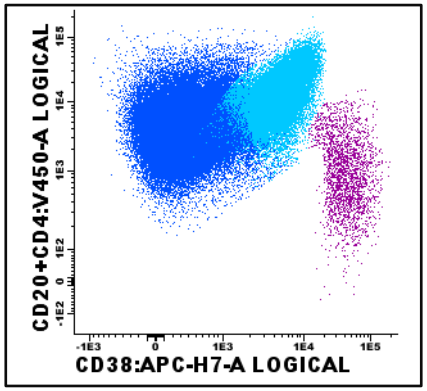


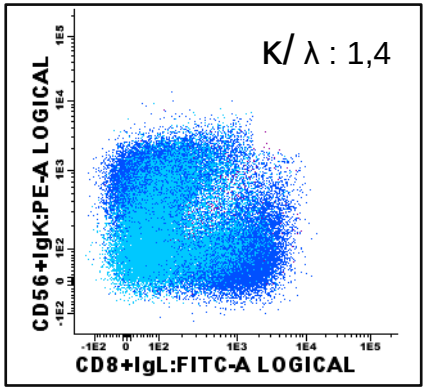


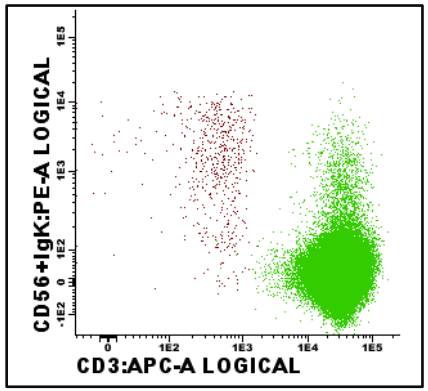






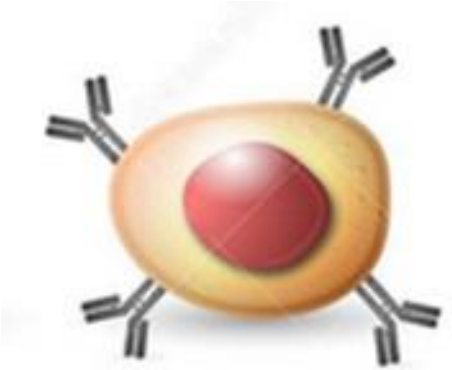


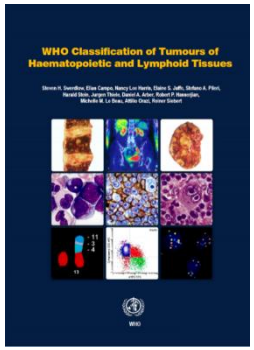




Linf T – Linf B policlonales – Linf B CG
Cel. NK - Cel.Plasmáticas

LINFOCITOS B



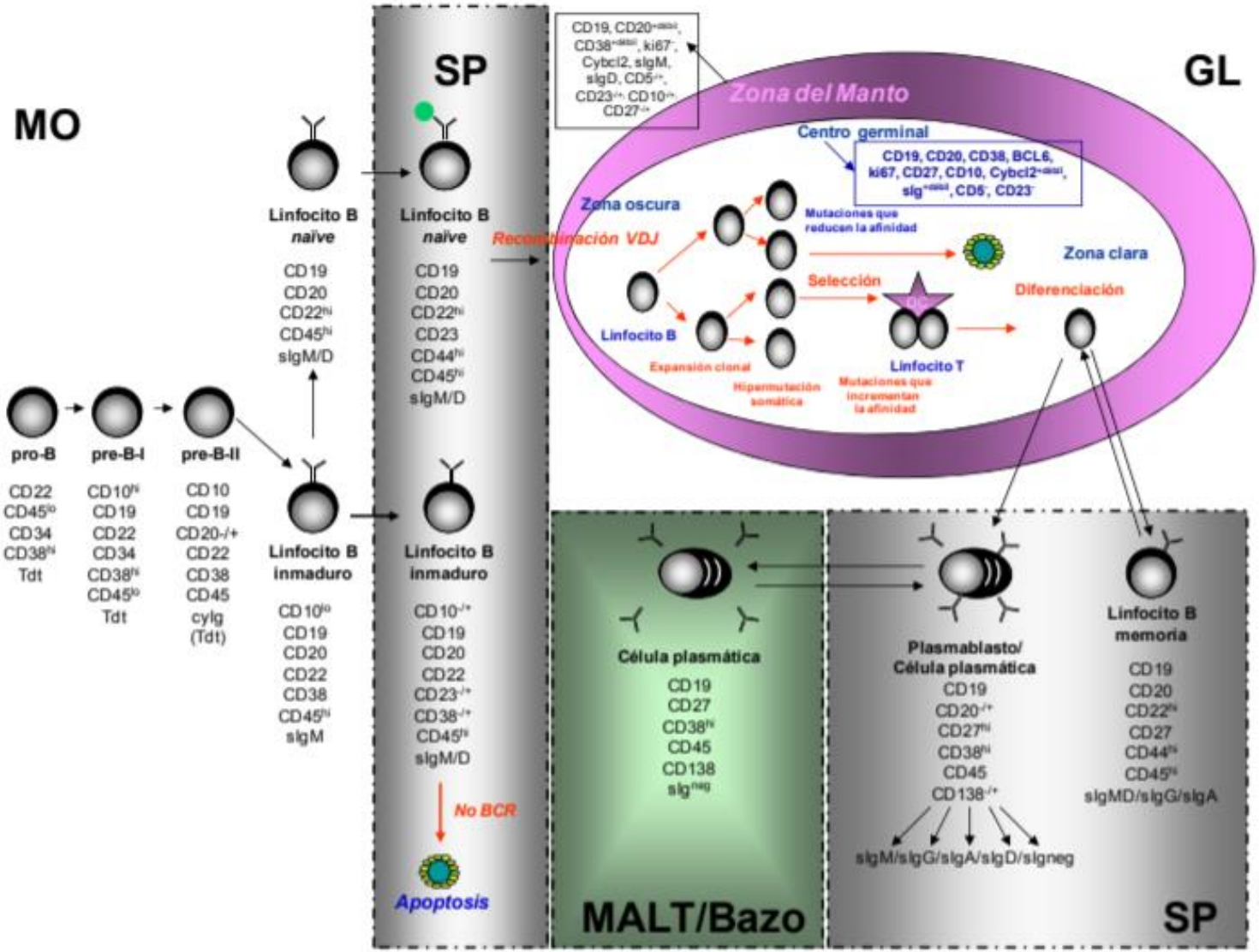


Neoplasia de Células B maduras

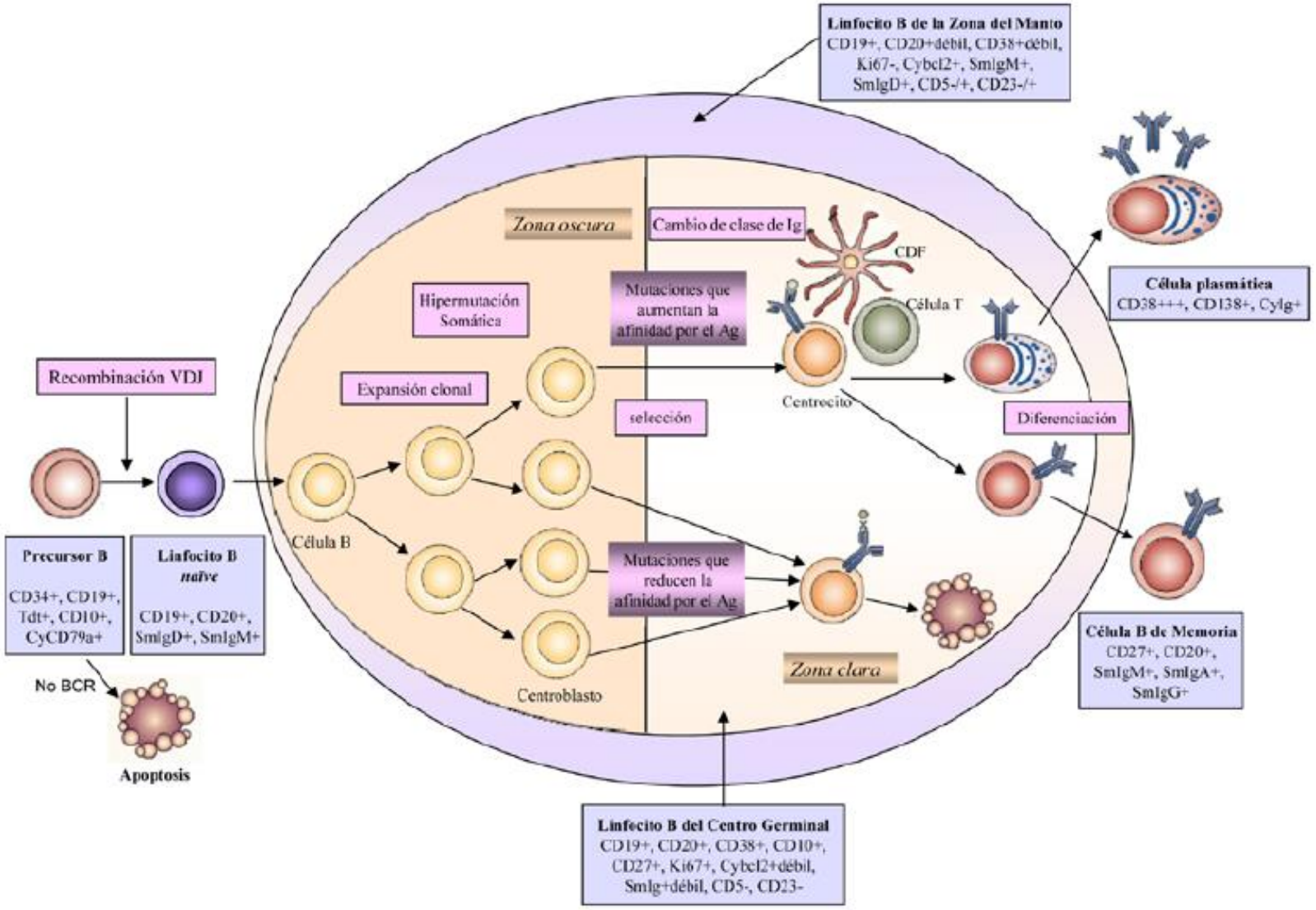
- Leucemia Linfocitica Crónica B/Linfoma de pequeños linfocitos B
- Leucemia Prolinfocítica de células B.
- Linfoma de zona marginal esplénico.
- Leucemia /linfoma esplénico inclasificable.
- Linfoma Linfoplasmocítico.
- Enfermedad de cadenas pesadas.
- Neoplasias de células plasmáticas.
- Linfoma de zona marginal extranodal de tejido linfoide asociado a mucosa (MALT).
- Linfoma de zona marginal nodal.
- Linfoma Folicular.
- Linfoma centro folicular primariamente cutáneo.
- Linfoma del Manto.
- Linfoma Difuso de Grandes Células B.
- Linfoma de Células B rico en Células T/histiocitos.
- Linfoma Plasmablástico.
- Linfoma de Burkitt.

Diferenciación linfoide B independiente de Ag

Diferenciación linfoide B dependiente de Ag

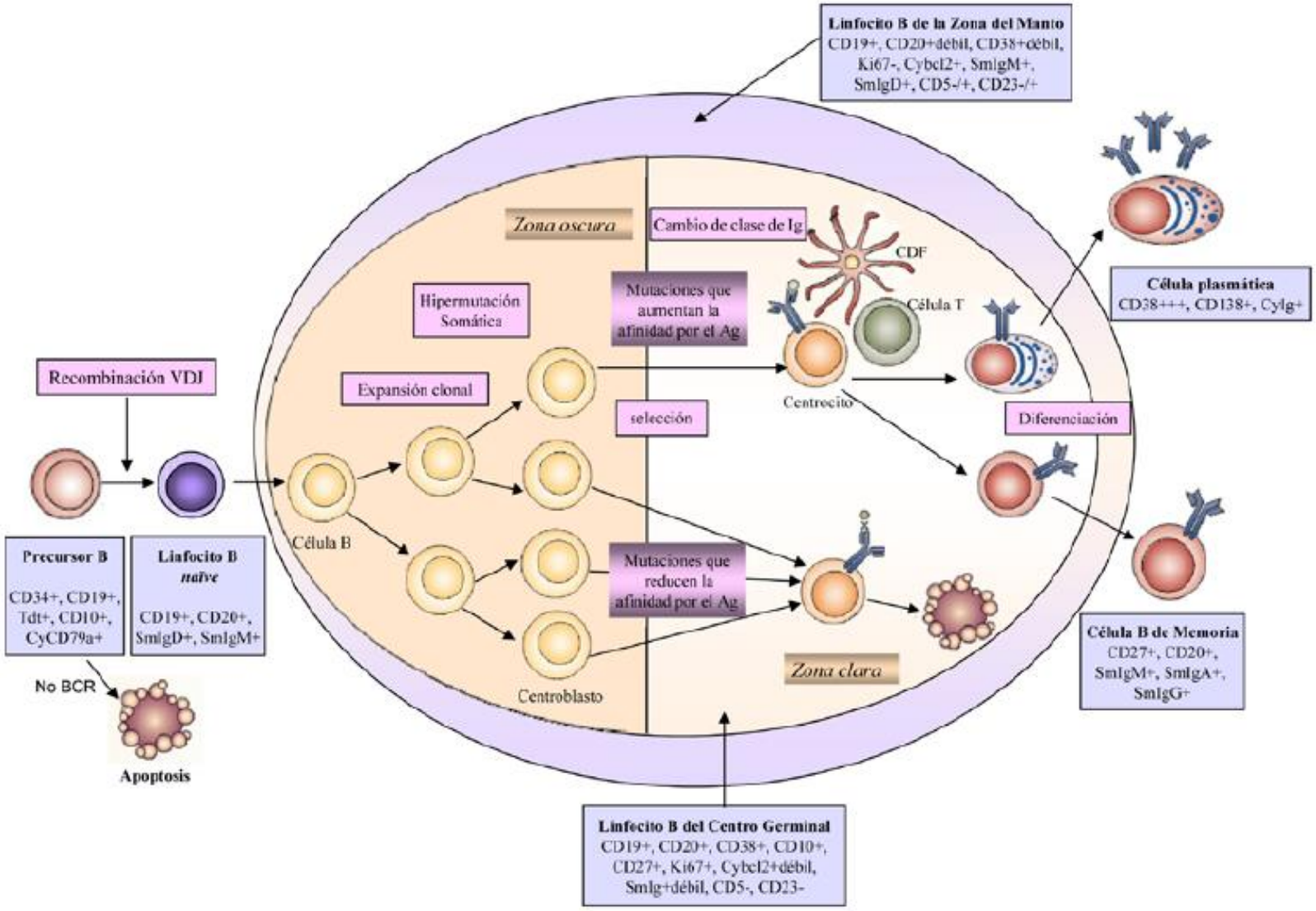


Diferenciación linfoide B en el Centro Germinal

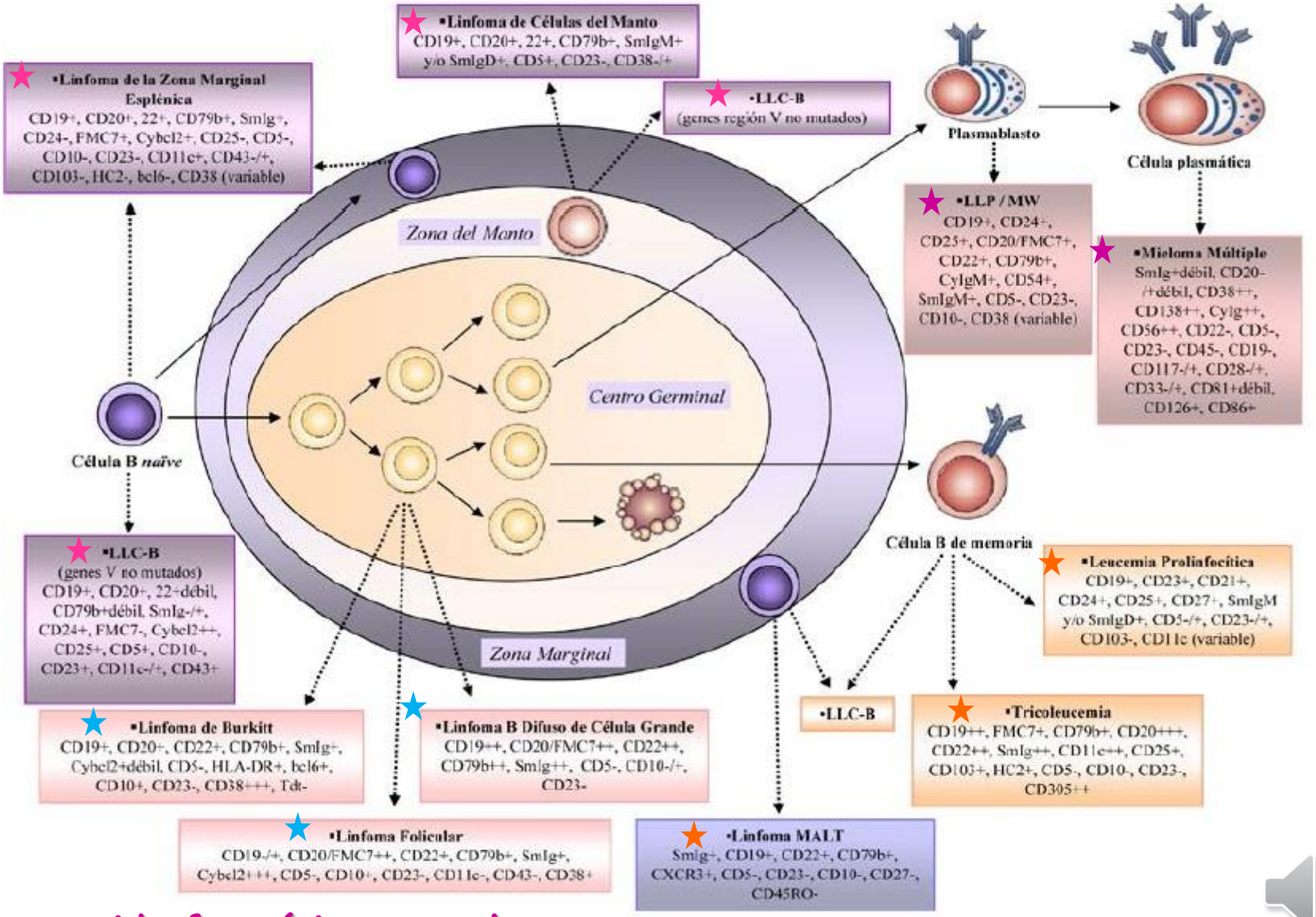


MORFOLOGÍA varía según la localización del tejido

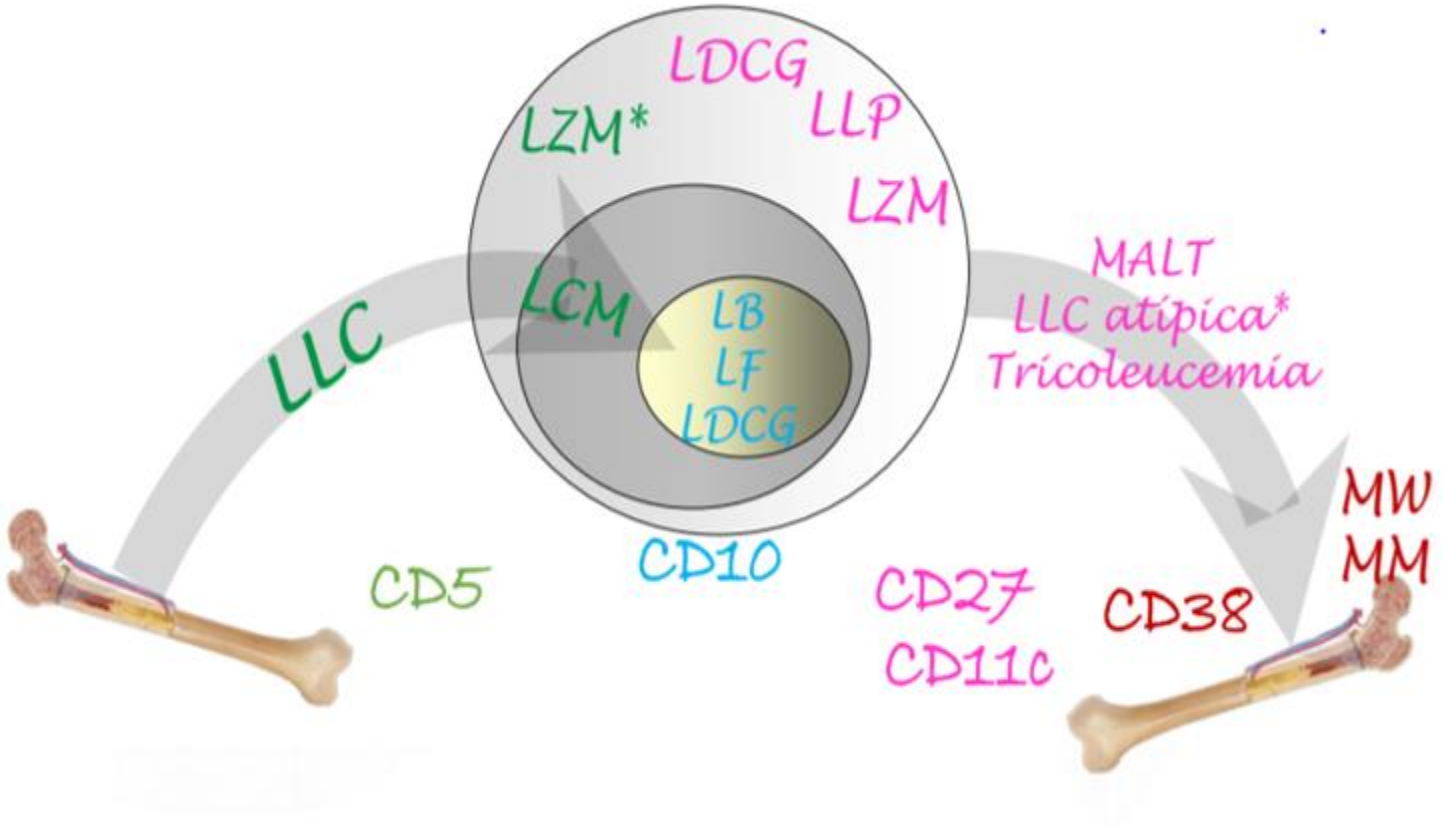
FENOTIPO varía según la localización del tejido



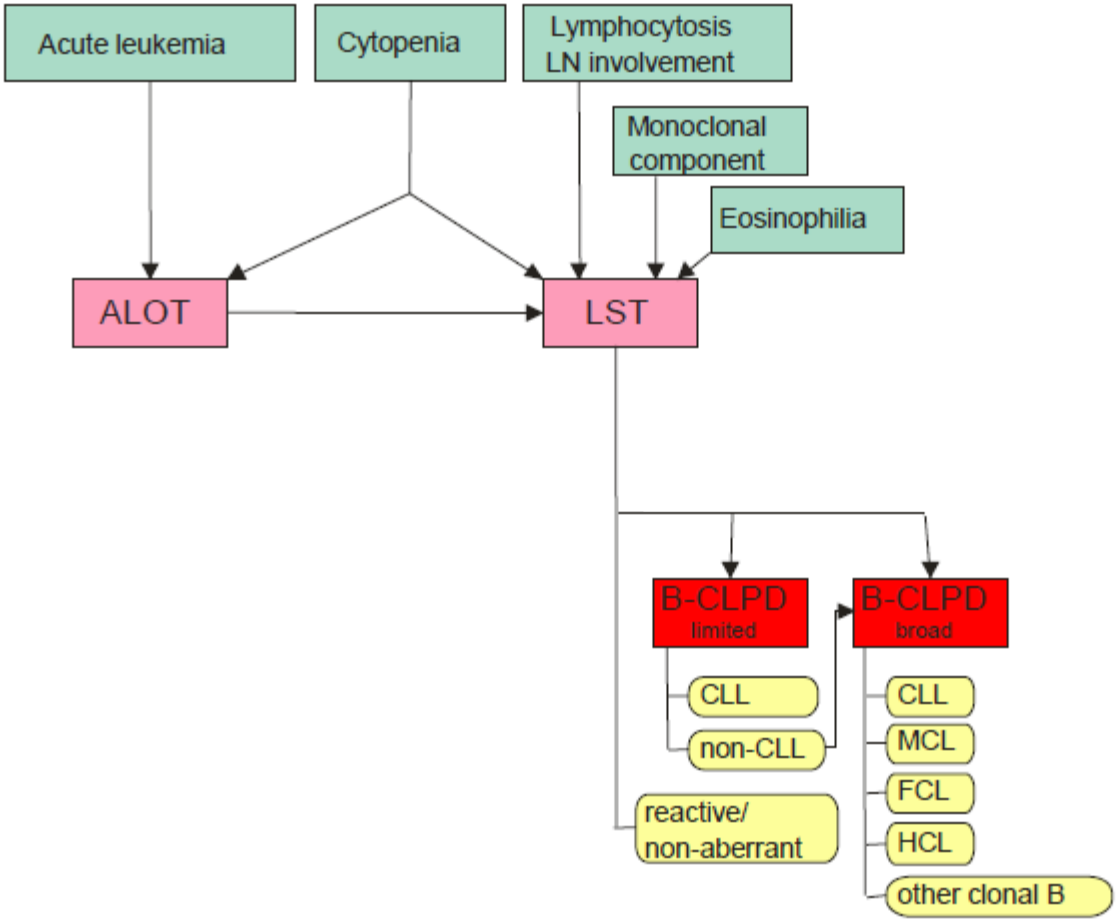
Origen celular de los SLPC-B


•Linfoma de Células del Manto
CD19+, CD20+, 22+, CD79b+, SmIgM+ y/o SmIgD+, CD5+, CD23-, CD38-/+
•Linfoma de la Zona Marginal Esplénica
CD19+, CD20+, 22+, CD79b+, SmIg+, CD24-, FMC7+, Cybcl2+, CD25-, CD5-, CD10-, CD23-, CD11c+, CD43-/+, CD103-, HC2-, bcl6-, CD38 (variable)
•LLC-B
(genes región V no mutados)
Plasmablasto
Célula plasmática
•LLP / MW
CD19+, CD24+, CD25+, CD20/FMC7+, CD22+, CD79b+, CylgM+, CD54+, SmIgM+, CD5-, CD23-, CD10-, CD38 (variable)
•Mieloma Múltiple
SmIg+débil, CD20-/+débil, CD38++, CD138++, Cylg++, CD56++, CD22-, CD5-, CD23-, CD45-, CD19-, CD117-/+, CD28-/+, CD33-/+, CD81+débil, CD126+, CD86+
Zona del Manto
Centro Germinal
Zona Marginal
Célula B naïve
Célula B de memoria
•LLC-B
(genes V no mutados)
CD19+, CD20+, 22+débil, CD79b+débil, SmIg-/+, CD24+, FMC7-, Cybcl2++, CD25+, CD5+, CD10-, CD23+, CD11c-/+, CD43+
•Leucemia Prolinfocítica
CD19+, CD23+, CD21+, CD24+, CD25+, CD27+, SmIgM y/o SmIgD+, CD5-/+, CD23-/+, CD103-, CD11c (variable)
•Linfoma de Burkitt
CD19+, CD20+, CD22+, CD79b+, SmIg+, Cybcl2+débil, CD5-, HLA-DR+, bcl6+, CD10+, CD23-, CD38+++, Tdt-
•Linfoma B Difuso de Célula Grande
CD19++, CD20/FMC7++, CD22++, CD79b++, SmIg++, CD5-, CD10-/+, CD23-
•LLC-B
•Tricoleucemia
CD19++, FMC7+, CD79b+, CD20+++, CD22++, SmIg++, CD11c++, CD25+, CD103+, HC2+, CD5-, CD10-, CD23-, CD305++
•Linfoma Folicular
CD19-/+, CD20/FMC7++, CD22+, CD79b+, SmIg+, Cybcl2+++, CD5-, CD10+, CD23-, CD11c-, CD43-, CD38+
•Linfoma MALT
SmIg+, CD19+, CD22+, CD79b+, CXCR3+, CD5-, CD23-, CD10-, CD27-, CD45RO-

Contrapartida fenotípica normal


LDCG
LZM*
LLP
LZM
LCM
LB
LF
LDCG
LLC
MALT
LLC atípica*
Tricoleucemia
MW
MM
CD5
CD10
CD27
CD11c
CD38

SLP-B: Algorítmo diagnóstico



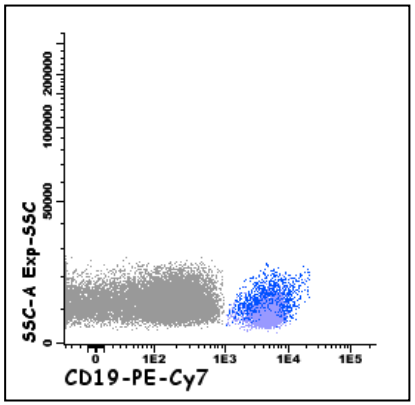
LST(Tubo de Screening Linfoide)

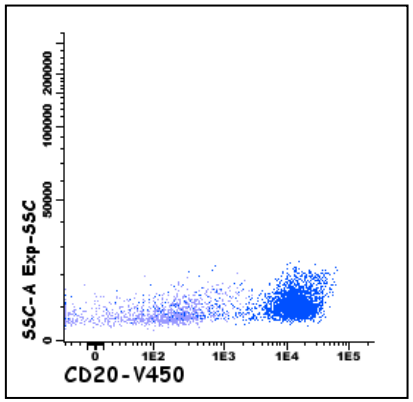
Pac Blue	Pac Orange	FITC	PE	PerCP Cy5.5	PE Cy7	APC	APC H7
CD20 CD4	CD45	Lambda CD8	Kappa CD56	CD5	CD19 TCR γδ	CD3	CD38

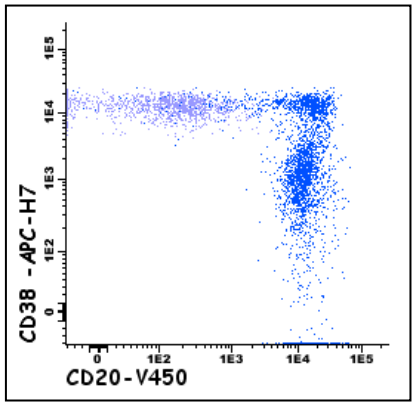
Linfocitos B

*Identificar linaje B: CD20, CD19 y CD38. Aberraciones B más comunes: CD5, y estudiar clonalidad: Lambda y Kappa.

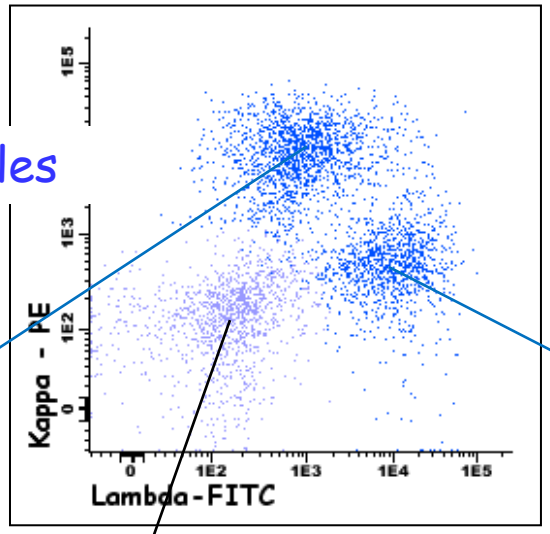
Estudiar clonalidad

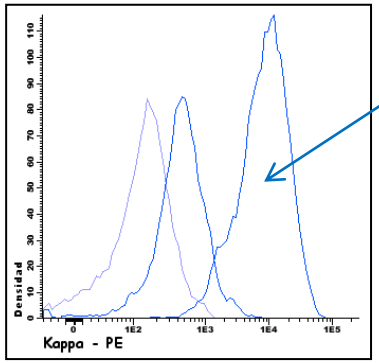


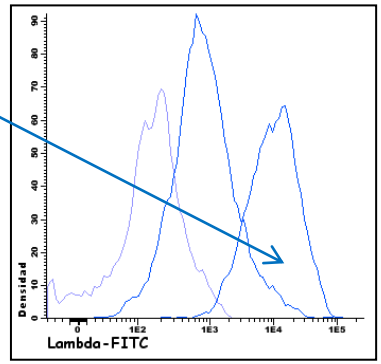




Linfocitos B Policlonales

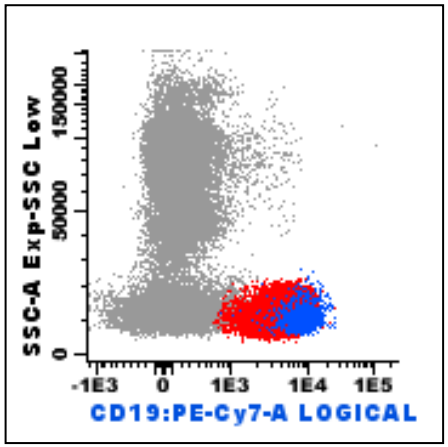


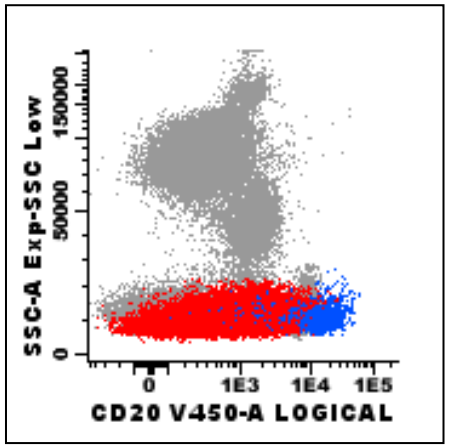


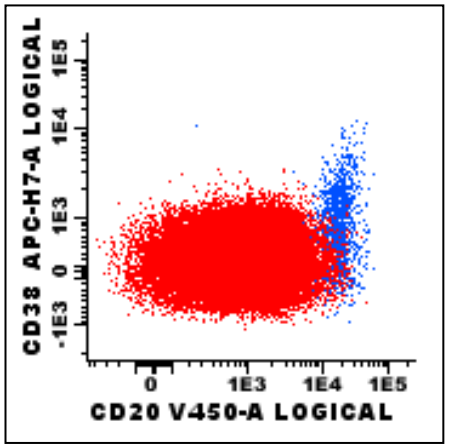


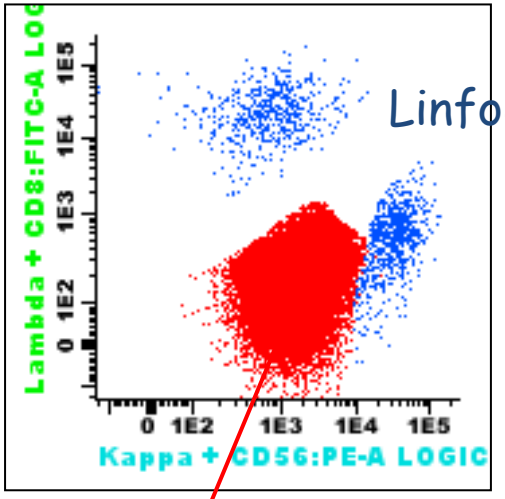
Linfocitos precursores B

Linfocitos clonales

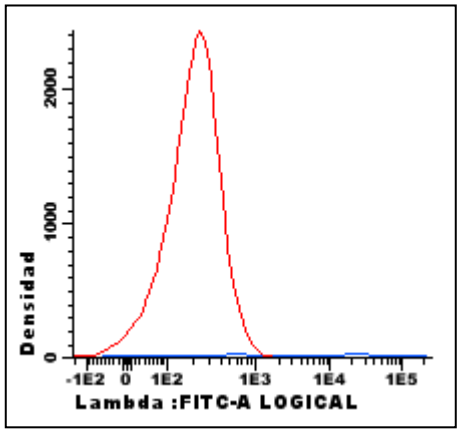




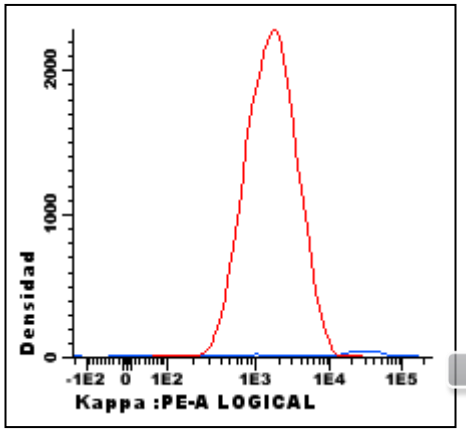




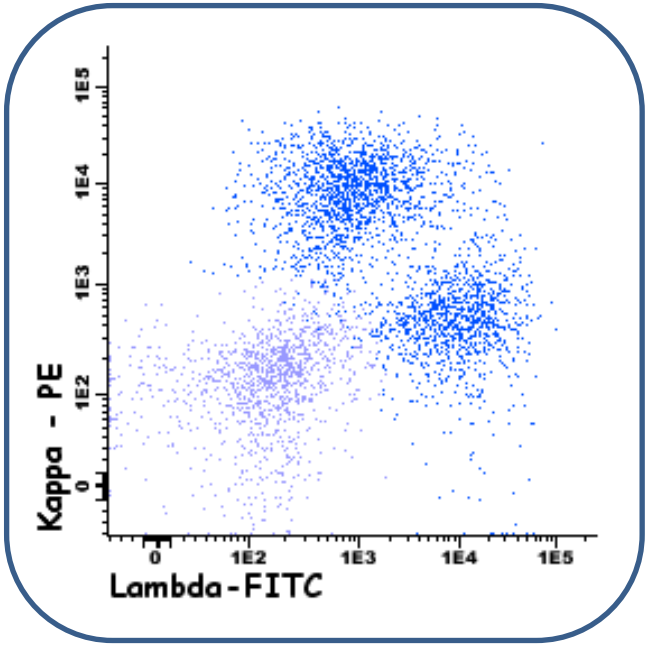
Linfocitos B Policlonales



Linfocitos B Clonales



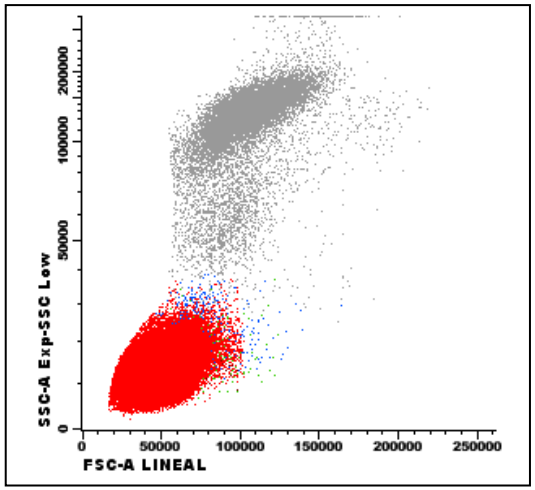
Estudiar clonalidad

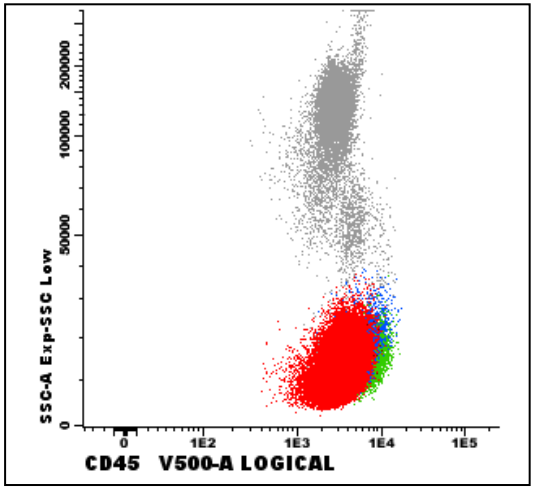


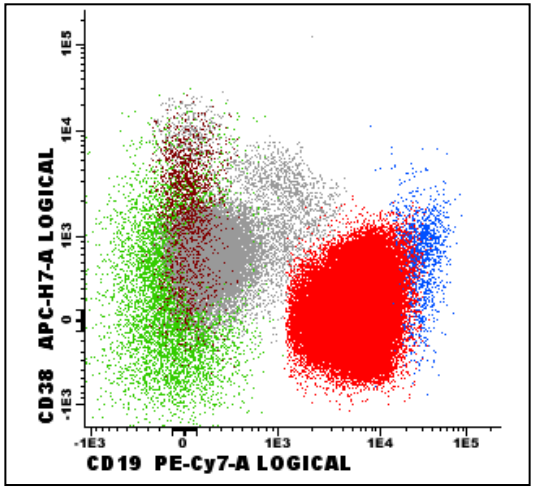
Relación sIgκ+/ sIgλ+ ALTERADA <0,3 ó > 3

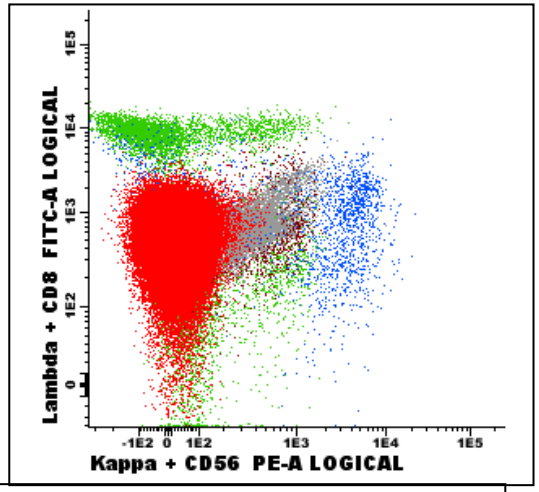
Identificación de fenotipos aberrantes

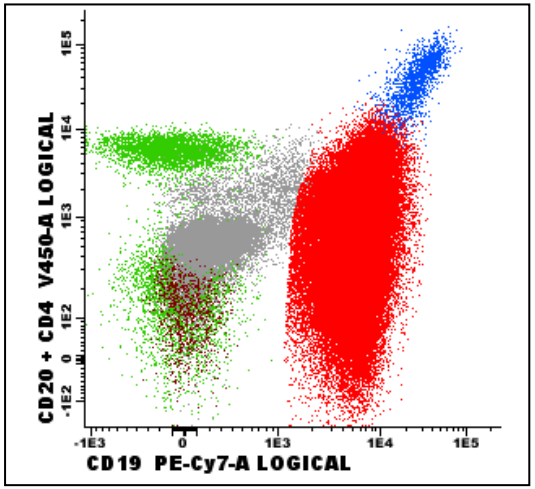
CD8-**sIglambda**/CD56-**sIgkappa**/**CD5**/**CD19**-TCRγδ/CD3/**CD38**/CD4-**CD20**/**CD45**

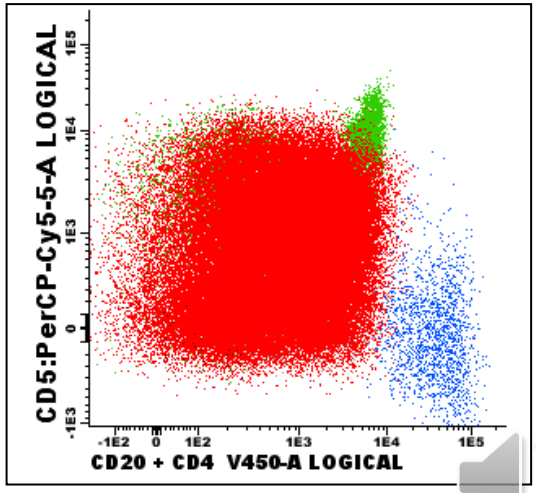












Linfocitos B LINFOCITOS B CLONALES
Linfocitos T Células NK

Panel para SLP-B

TABLE 8. Multi-tube EuroFlow classification combinations for B-CLPD *

Tube	Pacific Blue	Pacific Orange	FITC	PE	PerCP-Cy5.5	PE-Cy7	APC	APC-H7	Aim **
1	**CD20** and CD4	**CD45**	CD8 and smIgλ	CD56 and smIgκ	CD5	**CD19** and TCRγδ	smCD3	CD38	LST tube; detection of B-CLPD
2	**CD20**	**CD45**	CD23	CD10	CD79b	**CD19**	CD200	CD43	Identification of CLL vs other B-CLPD cases, when combined with LST
3	**CD20**	**CD45**	CD31	LAIR1	CD11c	**CD19**	smIgμ	CD81	Further subclassification of non-CLL B-CLPD, e.g. HCL, MCL, FL, MZL, LPL, DLBCL and other B-CLPD
4	**CD20**	**CD45**	CD103	CD95	CD22	**CD19**	CXCR5	CD49d	
5	**CD20**	**CD45**	CD62L	CD39	HLADR	**CD19**	CD27		

*Further information about the markers and the availability of hybridoma clones is summarized in Appendix A. Backbone markers are indicated in bold; sm= surface membrane. ** The described marker combinations might also be applied for disease staging and monitoring of treatment effectiveness (MRD diagnostics)

EuroFlow

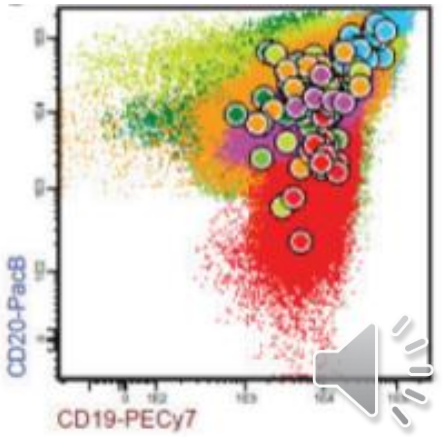
Marcadores vertebrales

TABLE 8. Multi-tube EuroFlow classification combinations for B-CLPD *

Tube	Pacific Blue	Pacific Orange	FITC	PE	PerCP-Cy5.5	PE-Cy7	APC	APC-H7	Aim **
1	CD20 and CD4	CD45	CD8 and smIgλ	CD56 and smIgκ	CD5	CD19 and TCRγδ	smCD3	CD38	LST tube; detection of B-CLPD
2	CD20	CD45	CD23	CD10	CD79b	CD19	CD200	CD43	Identification of CLL vs other B-CLPD cases, when combined with LST
3	CD20	CD45	CD31	LAIR1	CD11c	CD19	smIgμ	CD81	Further subclassification of non-CLL B-CLPD, e.g. HCL, MCL, FL, MZL, LPL, DLBCL and other B-CLPD
4	CD20	CD45	CD103	CD95	CD22	CD19	CXCR5	CD49d	
5	CD20	CD45	CD62L	CD39	HLADR	CD19	CD27		

- Identificar a todas las células B.

- Se testeó la expresión de λ/CD37/k/CD19/CD22/CD20 en 69 casos de SLP-B.



Identificación

FSC SSC
CD19 CD20 CD81 CD79b
sIgKappa sIgLambda

Maduración

CD5 CD10
CD38 IgM
HLA DR CD22
CD23 CD27

Activación/Interacción

CD39
CD43
CD200

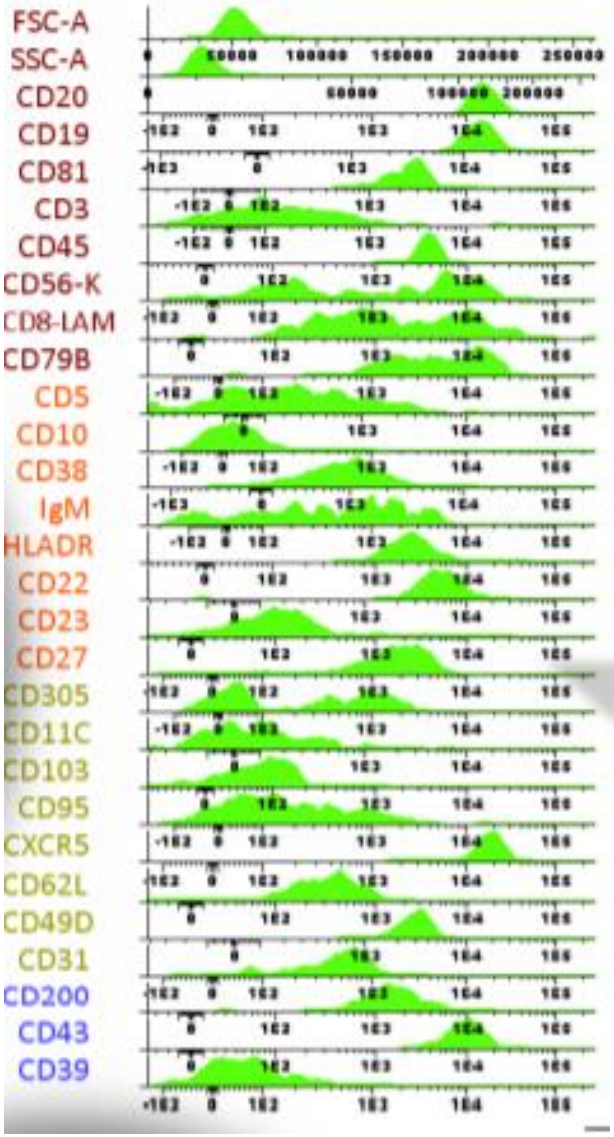
Migración /Homing

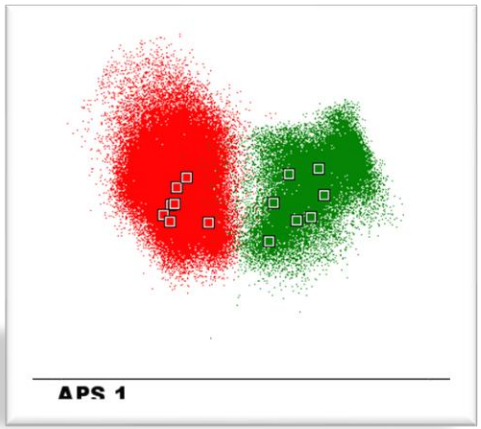
CD305 CD11c
CD95 CXCR5
CD31 CD49d
CD62L

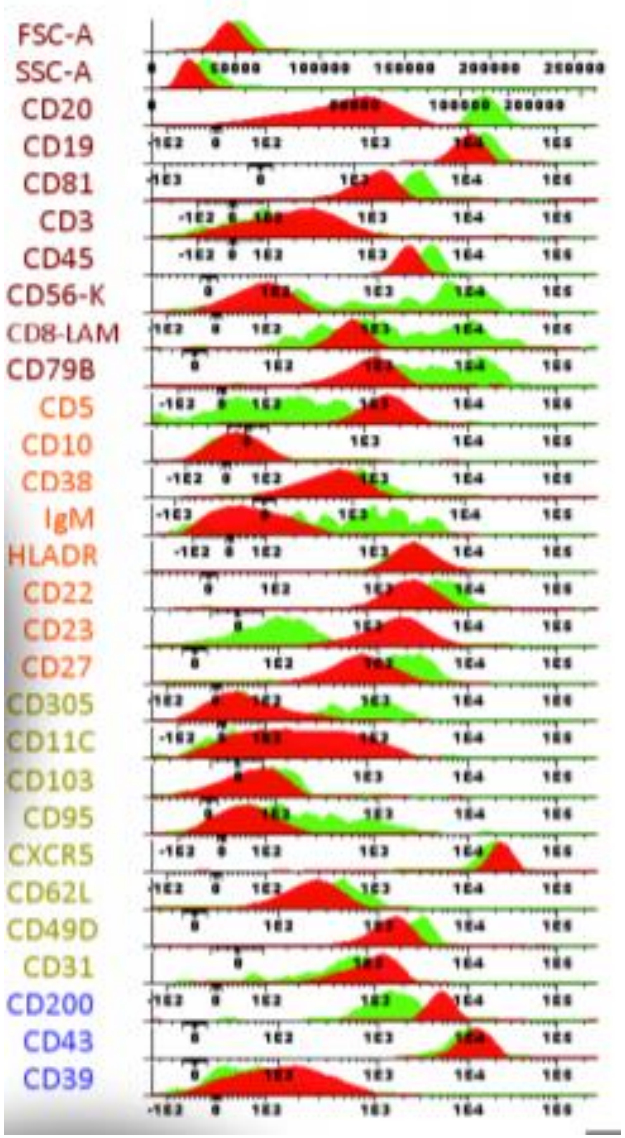
EuroFlow

TABLE 8. Multi-tube EuroFlow classification combinations for B-CLPD *

Tube	Pacific Blue	Pacific Orange	FITC	PE	PerCP-Cy5.5	PE-Cy7	APC	APC-H7	Aim **
1	CD20 and CD4	CD45	CD8 and smIgλ	CD56 and smIgκ	CD5	CD19 and TCRγδ	smCD3	CD38	LST tube; detection of B-CLPD
2	CD20	CD45	CD23	CD10	CD79b	CD19	CD200	CD43	LLC vs otros SLP-B (LCM)
3	CD20	CD45	CD31	LAIR1	CD11c	CD19	smIgμ	CD81	Tricoleucemia (HCL)
4	CD20	CD45	CD103	CD95	CD22	CD19	CXCR5	CD49d	Burkitt HCL
5	CD20	CD45	CD62L	CD39	HLADR	CD19	CD27		Centro germinal







Linfocitos B normales

Linfocitos B clonales

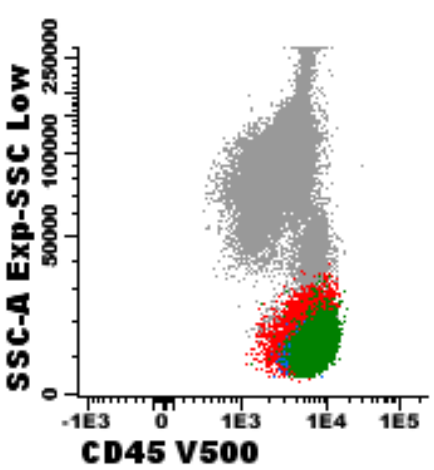
Muestra: Sangre periférica

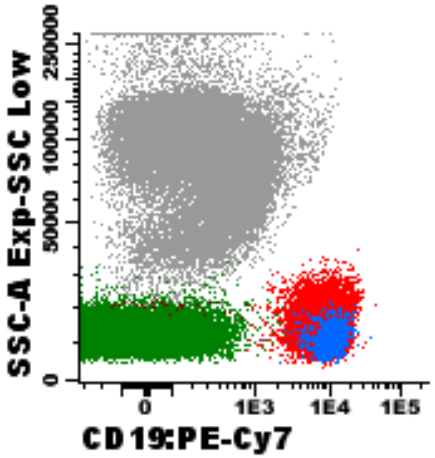
LLC-B

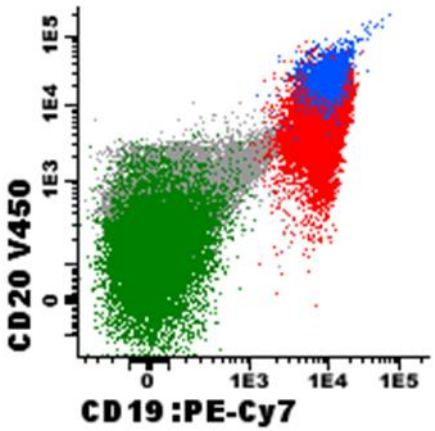
1-LST

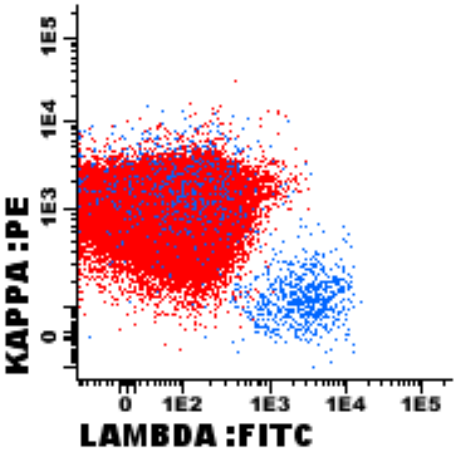
Pac Blue	Pac Orange	FITC	PE	PerCP Cy5.5	PE Cy7	APC	APC H7
CD20 CD4	CD45	Lambda CD8	Kappa CD56	CD5	CD19 TCR γδ	CD3	CD38

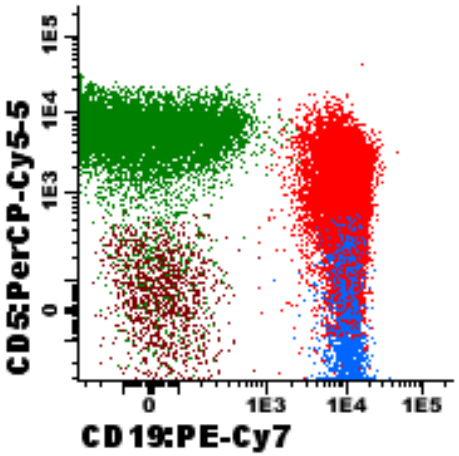
Linfocitos B Linfocitos T Celulas NK
LINFOCITOS B CLONALES

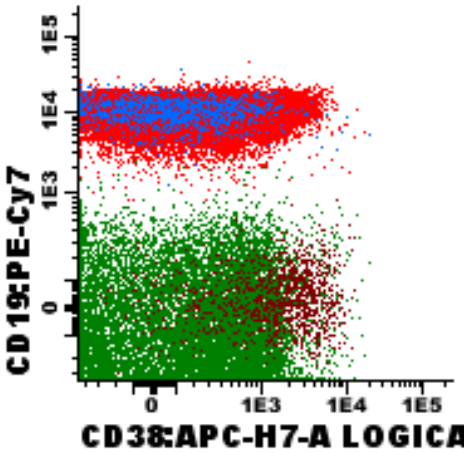




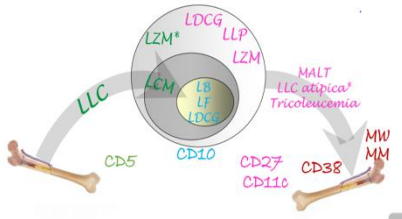






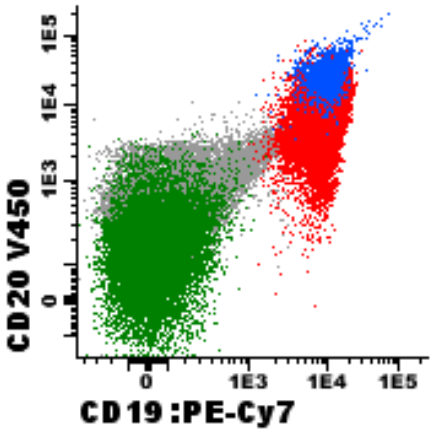


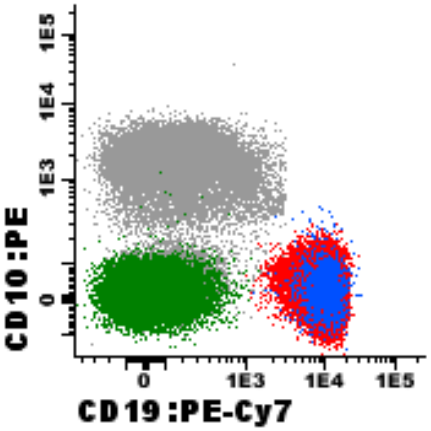
Linfoma Pre CG

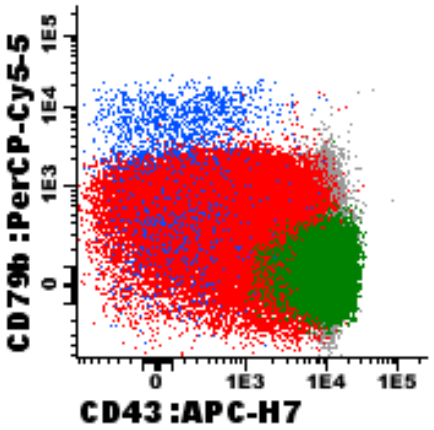


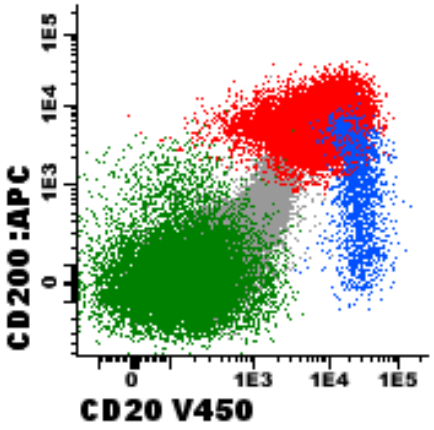
Linfocitos B Linfocitos T Celulas NK
LINFOCITOS B CLONALES

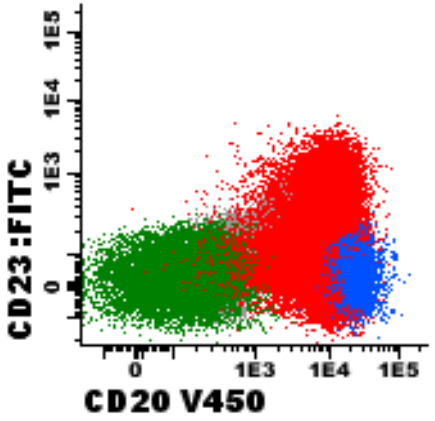
LLC-B

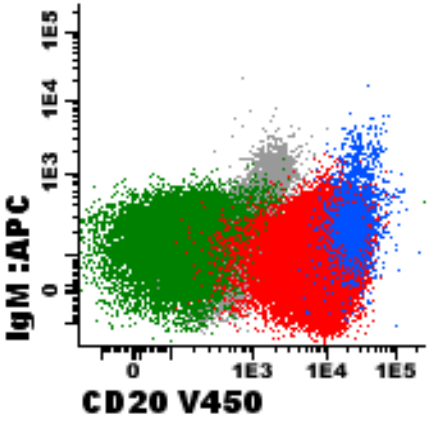


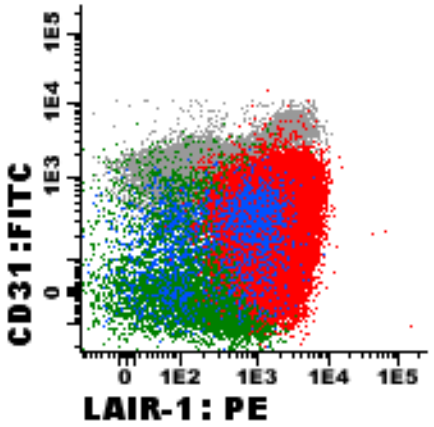


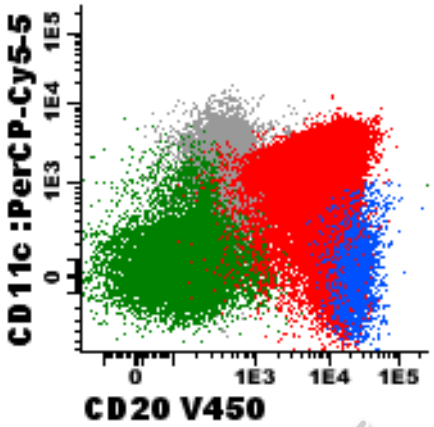








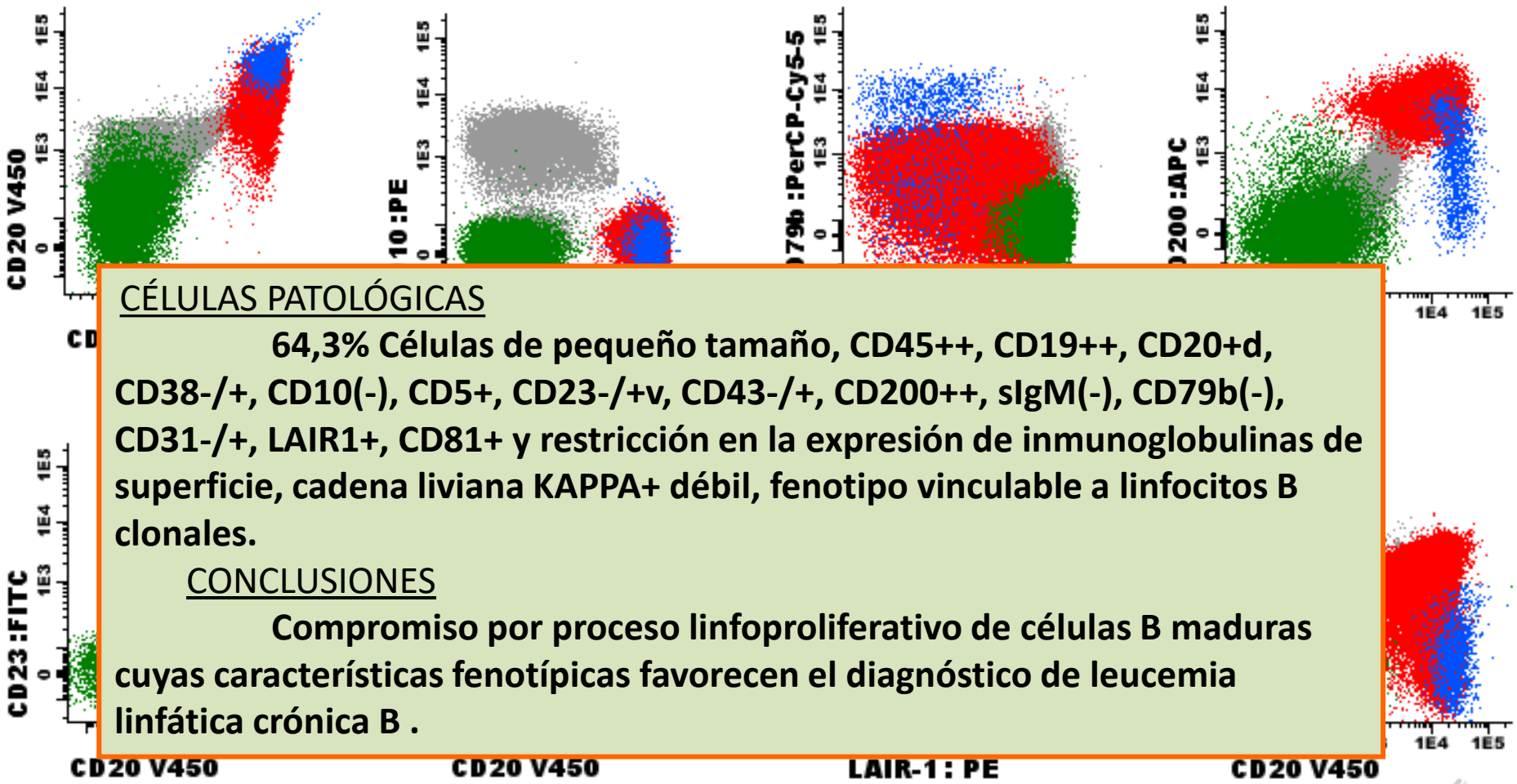




Muestra: Sangre periférica

Linfocitos B Linfocitos T Celulas NK
LINFOCITOS B CLONALES

LLC-B



CÉLULAS PATOLÓGICAS

64,3% Células de pequeño tamaño, CD45++, CD19++, CD20+d, CD38-/+, CD10(-), CD5+, CD23-/+v, CD43-/+, CD200++, sIgM(-), CD79b(-), CD31-/+, LAIR1+, CD81+ y restricción en la expresión de inmunoglobulinas de superficie, cadena liviana KAPPA+ débil, fenotipo vinculable a linfocitos B clonales.

CONCLUSIONES

Compromiso por proceso linfoproliferativo de células B maduras cuyas características fenotípicas favorecen el diagnóstico de leucemia linfática crónica B .

Muestra: Sangre periférica

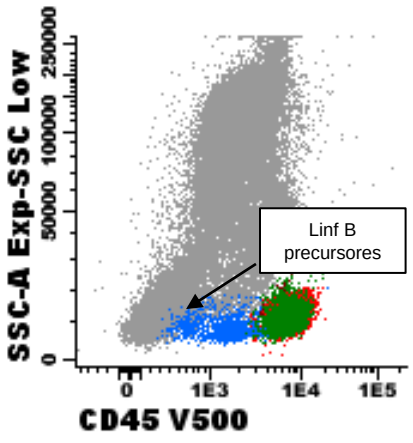
Linfoma del Manto

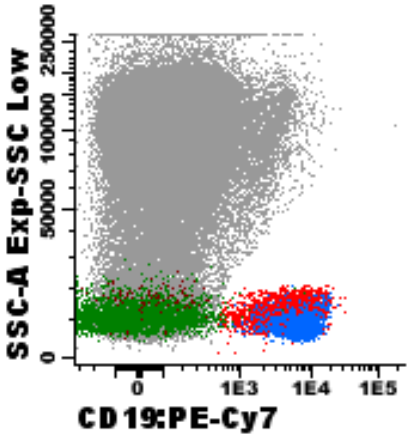
Muestra: Médula ósea

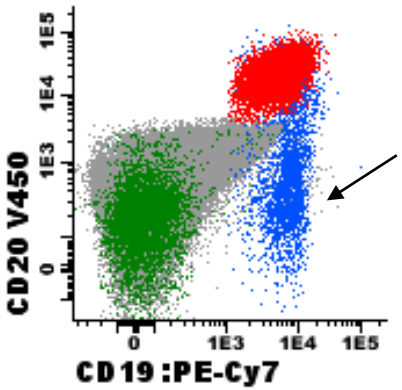
Linfocitos B Linfocitos T Celulas NK
LINFOCITOS B CLONALES

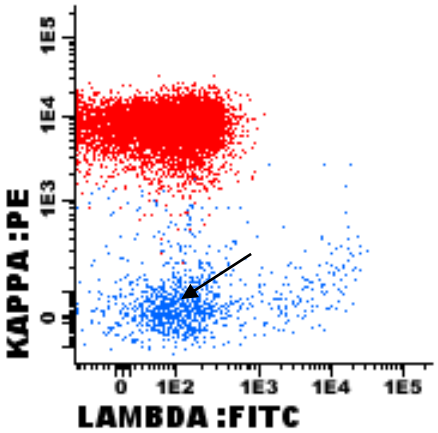
1-LST

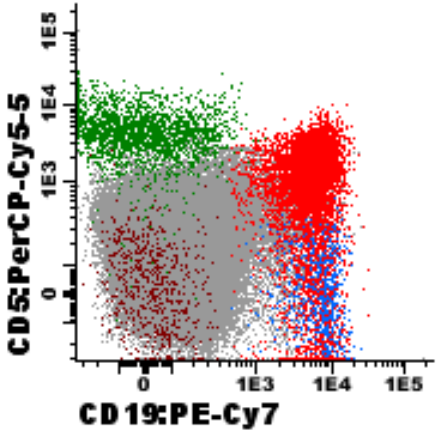
Pac Blue	Pac Orange	FITC	PE	PerCP Cy5.5	PE Cy7	APC	APC H7
CD20 CD4	CD45	Lambda CD8	Kappa CD56	CD5	CD19 TCR γδ	CD3	CD38

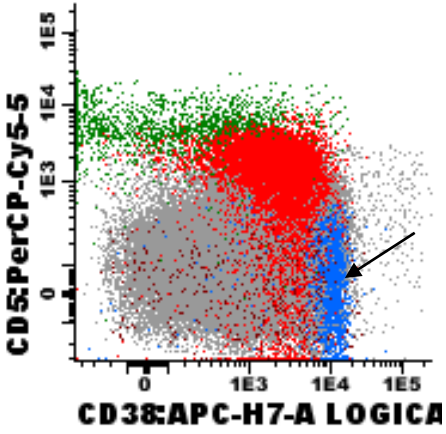




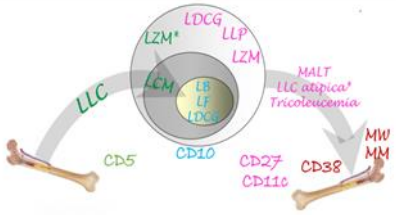








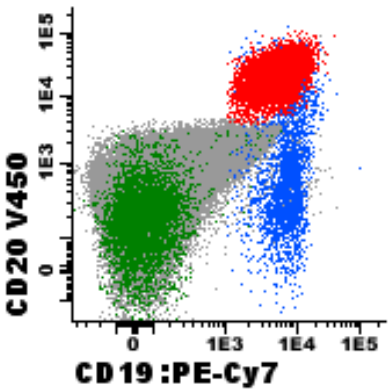
Linfoma Pre CG

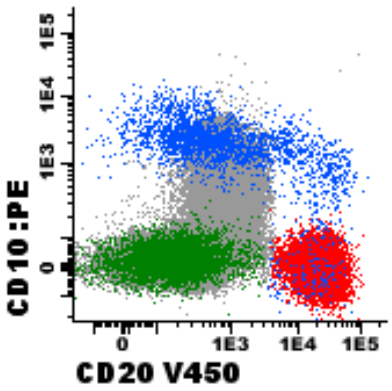


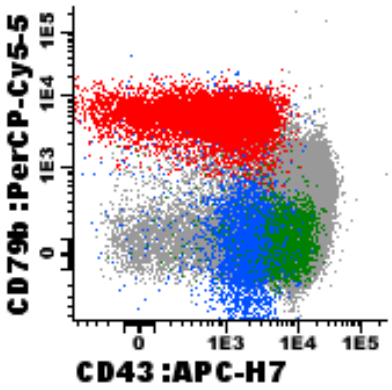
Linfoma del Manto

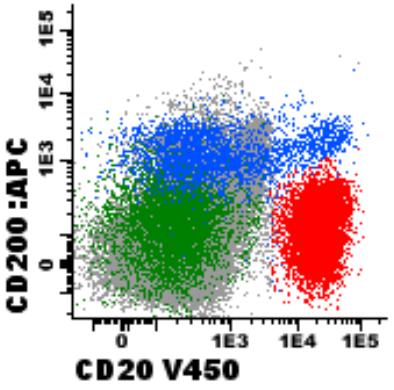
Linfocitos B Linfocitos T Celulas NK
LINFOCITOS B CLONALES

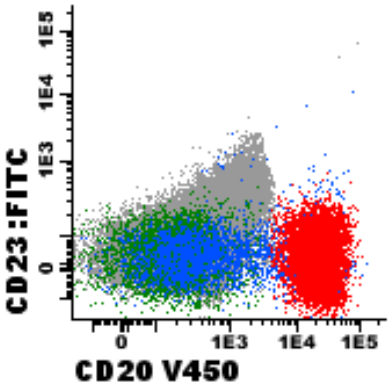
Muestra: Médula ósea

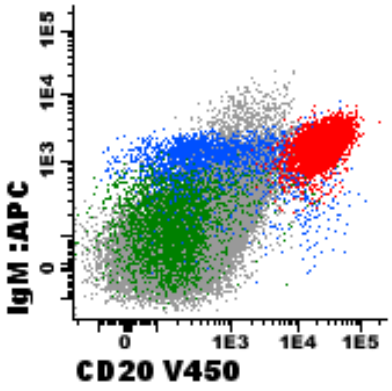


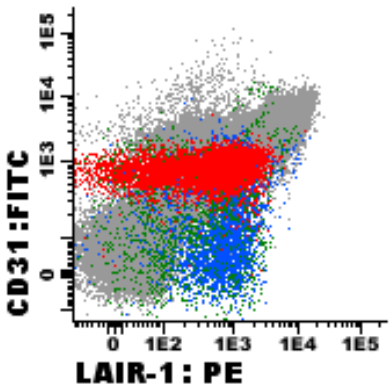


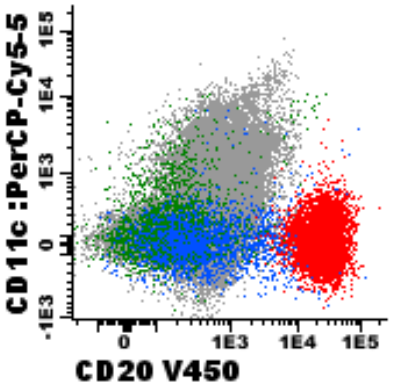








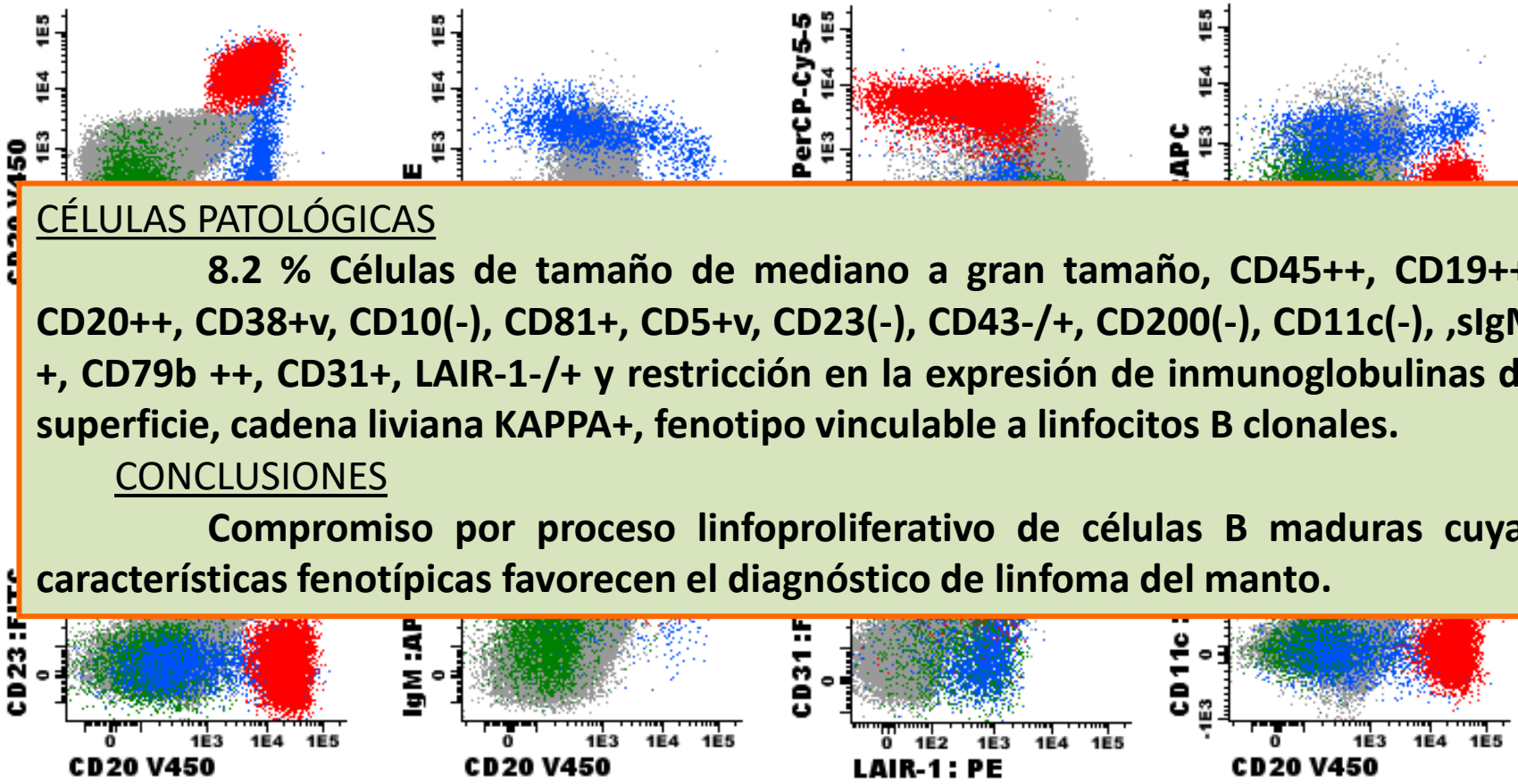




Linfoma del Manto

Linfocitos B Linfocitos T Celulas NK
LINFOCITOS B CLONALES

Muestra: Médula ósea

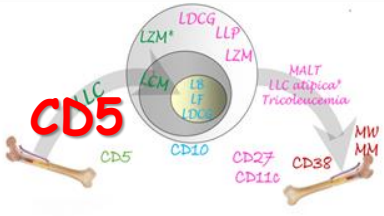


CÉLULAS PATOLÓGICAS

8.2 % Células de tamaño de mediano a gran tamaño, CD45++, CD19++, CD20++, CD38+v, CD10(-), CD81+, CD5+v, CD23(-), CD43-/+, CD200(-), CD11c(-), ,sIgM +, CD79b ++, CD31+, LAIR-1-/+ y restricción en la expresión de inmunoglobulinas de superficie, cadena liviana KAPPA+, fenotipo vinculable a linfocitos B clonales.

CONCLUSIONES

Compromiso por proceso linfoproliferativo de células B maduras cuyas características fenotípicas favorecen el diagnóstico de linfoma del manto.



LLC-B

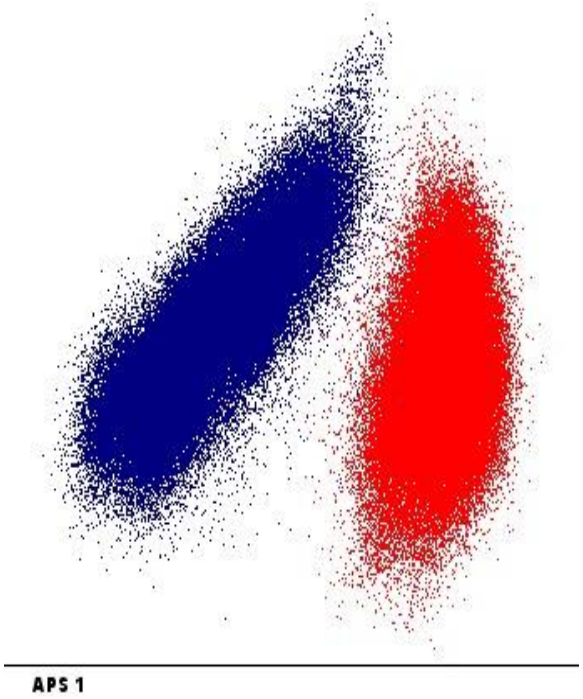
- ✓**Igs K ó λ débil**
- ✓CD19+
- ✓CD5+
- ✓**CD20 + débil**
- ✓CD43+
- ✓**CD23 +**
- ✓**CD200 +**
- ✓**CD79b (-)**
- ✓**IgM (-)**

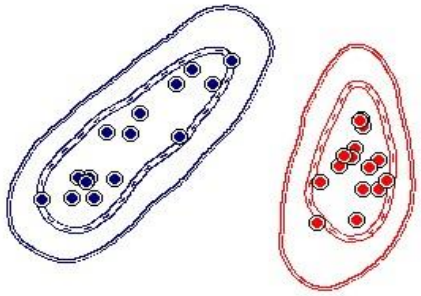
Linfoma del Manto

- ✓**Igs K ó λ int**
- ✓CD19+
- ✓CD5+
- ✓**CD20 +int**
- ✓CD43+
- ✓**CD23 (-)**
- ✓**CD200 (-)**
- ✓**CD79b +**
- ✓**IgM +**

Utilidad de la CF para el diagnostico diferencial

LLC vs Linfoma del Manto





View 1

Parameter	Significance
PerCP Cy5-5-A:CD79b LOGICAL	13.15
APC-A:CD200 LOGICAL	12.88
APC-A:IgM LOGICAL	11.96
FITC-A:CD23 LOGICAL	7.16
PB-A:CD20 LOGICAL	7.06
APC H7-A:CD43 LOGICAL	6.13
PE-A:LAIR1 LOGICAL	4.60
PerCP Cy5-5-A:CD22 LOGICAL	4.26
FITC-A:CD62L LOGICAL	3.27
APC H7-A:CD38 LOGICAL	3.25

View 2

Parameter	Significance
APC H7-A:CD43 LOGICAL	8.10
APC-A:CD27 LOGICAL	7.92
FITC-A:CD23 LOGICAL	7.77
PE-A:CD56+IgK LOGICAL	6.97
APC-A:CD200 LOGICAL	6.70
PerCP Cy5-5-A:CD5 LOGICAL	6.01
PE-A:CD39 LOGICAL	5.19
FITC-A:CD62L LOGICAL	4.67
APC-A:CXCR5 LOGICAL	3.95
FITC-A:CD31 LOGICAL	3.78
APC H7-A:CD49d LOGICAL	3.32

Importancia:

CD79b 13,15

CD200 12,88

IgM 11,96

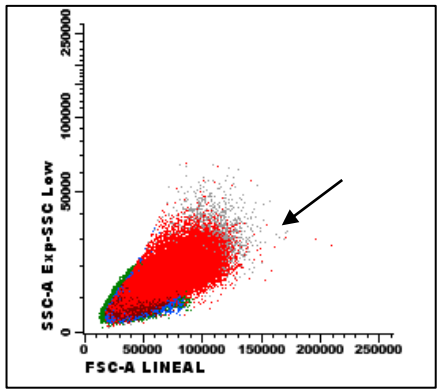
Linfoma Folicular

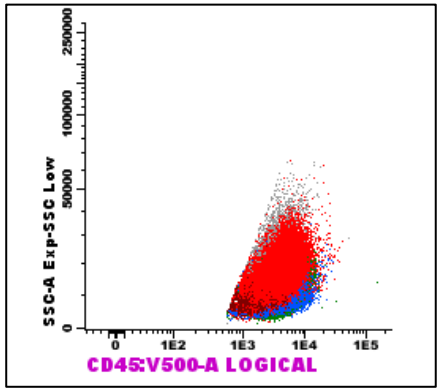
Linfocitos B Linfocitos T Celulas NK
LINFOCITOS B CLONALES

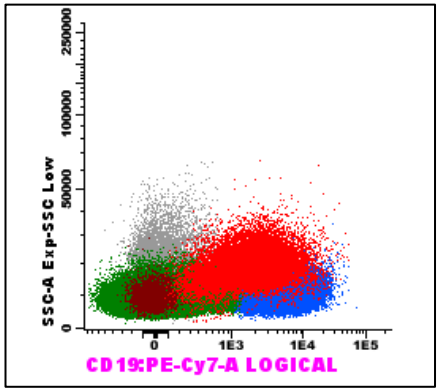
Muestra:GANGLIO

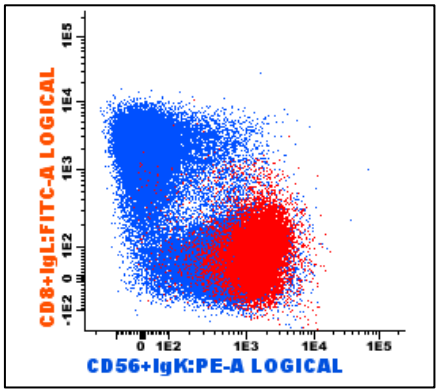
1-LST

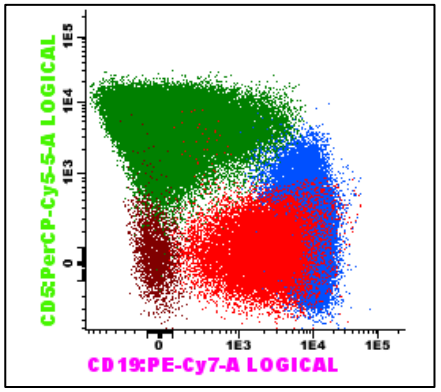
Pac Blue	Pac Orange	FITC	PE	PerCP Cy5.5	PE Cy7	APC	APC H7
CD20 CD4	CD45	Lambda CD8	Kappa CD56	CD5	CD19 TCR γδ	CD3	CD38

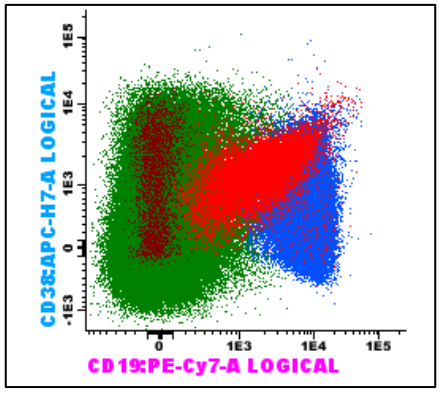


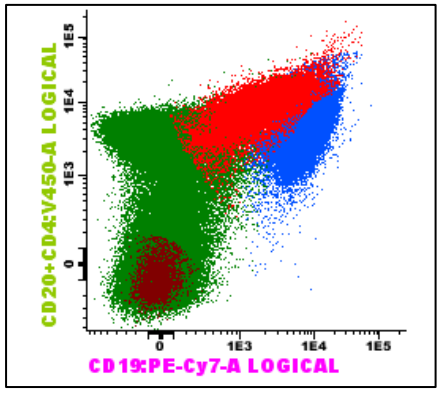








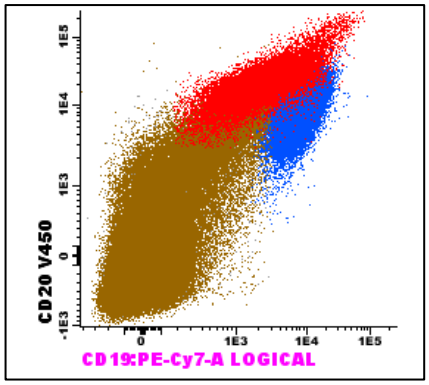


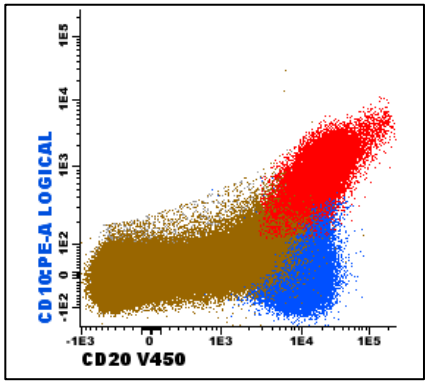


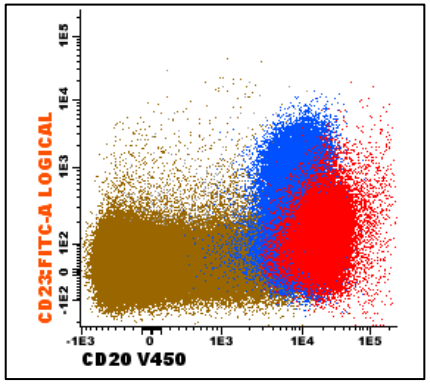
Linfoma Folicular

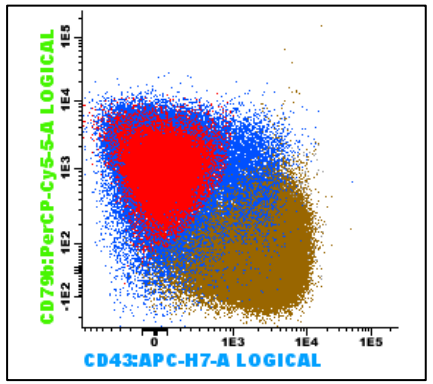
Linf. B policlonales Linf. B CLONALES
Otros linfocitos

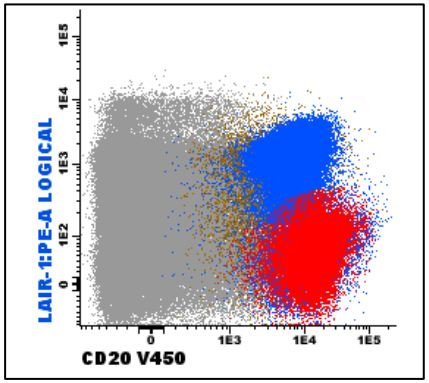
Muestra:GANGLIO

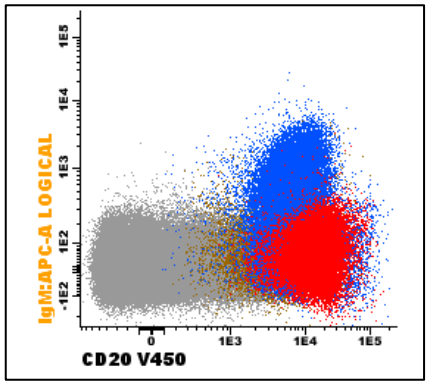


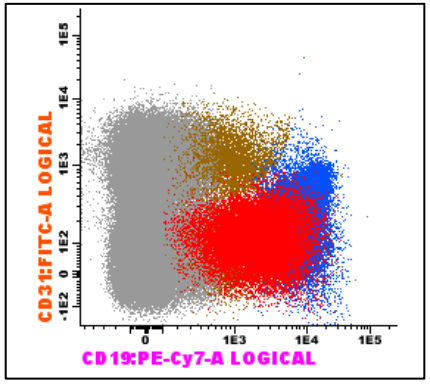


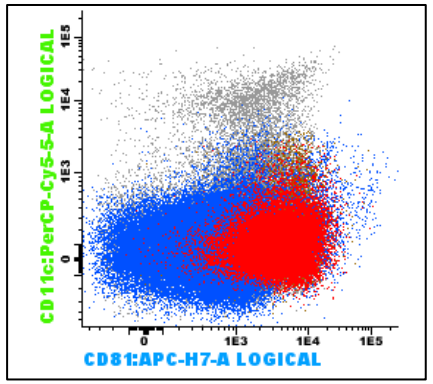




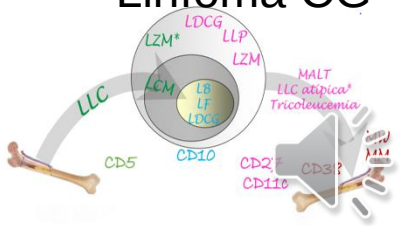








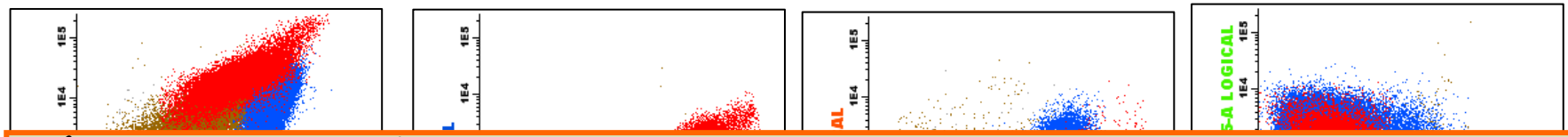
Linfoma CG



Linfoma Folicular

Linf. B policlonales Linf. B CLONALES
Otros linfocitos

Muestra:GANGLIO



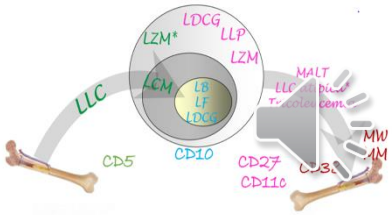
CÉLULAS PATOLÓGICAS

9,7 % Células de tamaño pequeño a mediano CD45++, CD19+v, CD20++, CD38+v, CD10+, CD5(-), CD23(-), CD43(-), CD200(-), CD11c(-), CD79b+, sIgM (-), CD31(-), LAIR 1(-), CD81+ y restricción en la expresión de inmunoglobulinas de superficie, cadena liviana KAPPA+, fenotipo vinculable a linfocitos B clonales.

CONCLUSIONES

Compromiso por proceso linfoproliferativo de células B maduras. Si bien la expresión de CD10 se asocia a origen centrofolicular no implica necesariamente patrón histológico; sin embargo las características fenotípicas globales favorecen el diagnóstico de linfoma folicular.

Linfoma CG



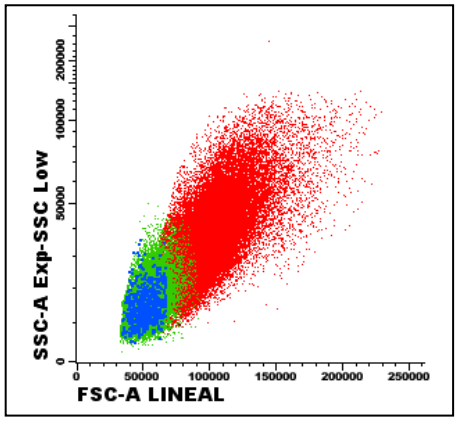
Linfoma comp. de grandes células

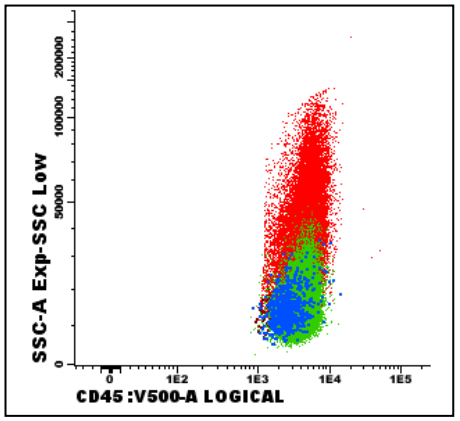
Linf. T
Linf. B policlonales
Linf. B patológicos

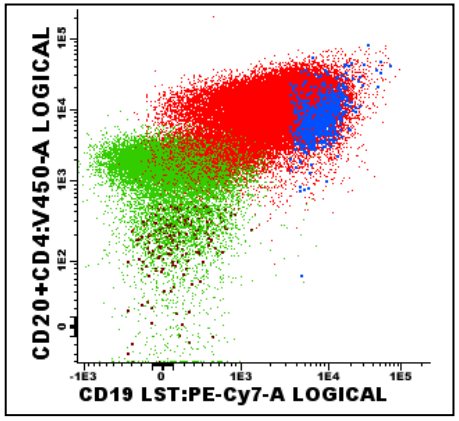
Muestra: Ganglio

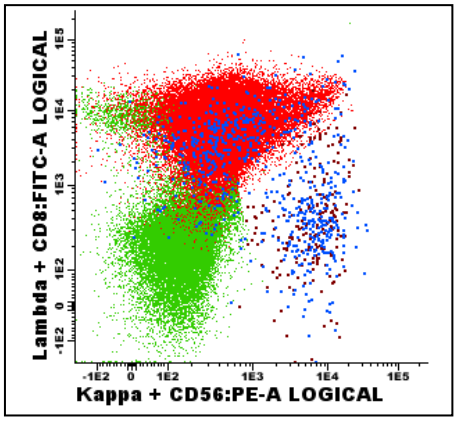
1-LST

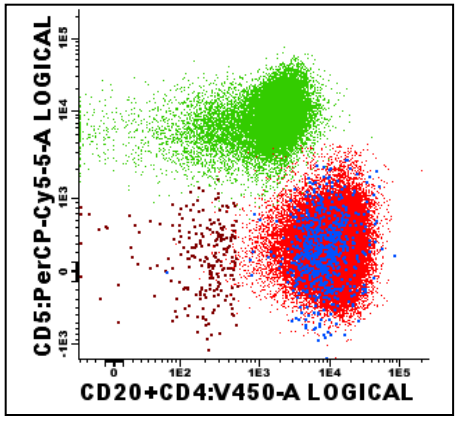
Pac Blue	Pac Orange	FITC	PE	PerCP Cy5.5	PE Cy7	APC	APC H7
CD20 CD4	CD45	Lambda CD8	Kappa CD56	CD5	CD19 TCR γδ	CD3	CD38

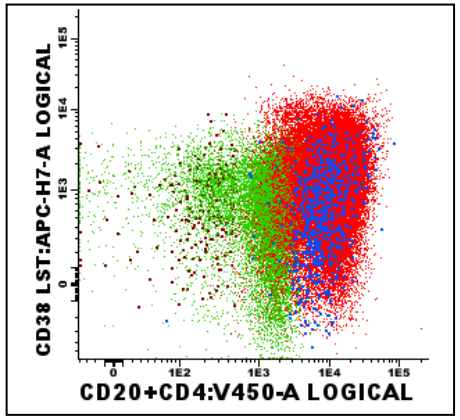




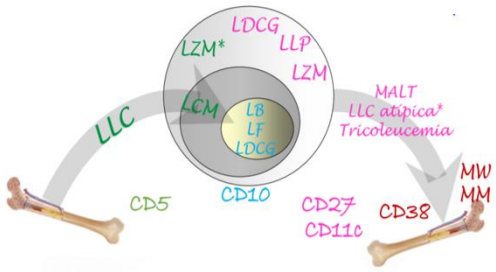








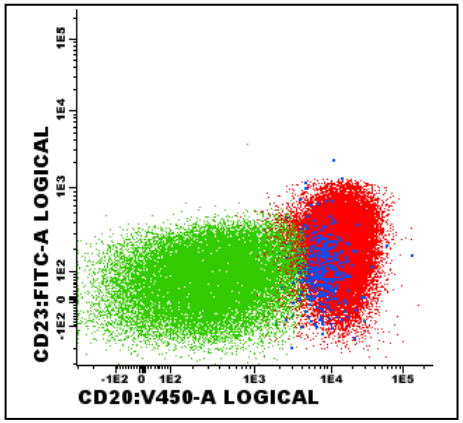
Linfoma CG

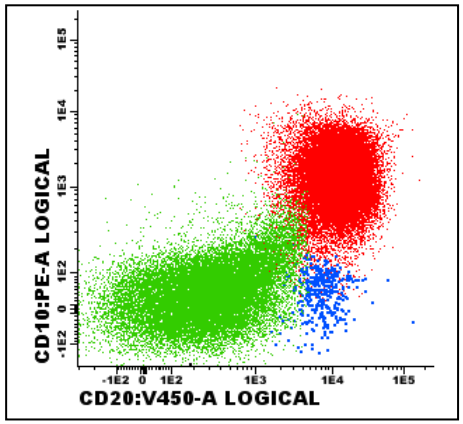


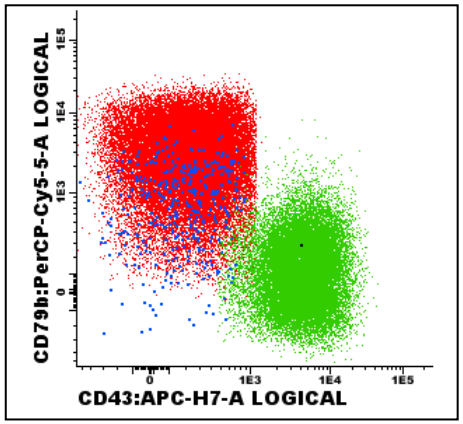
Linfoma comp. de grandes células

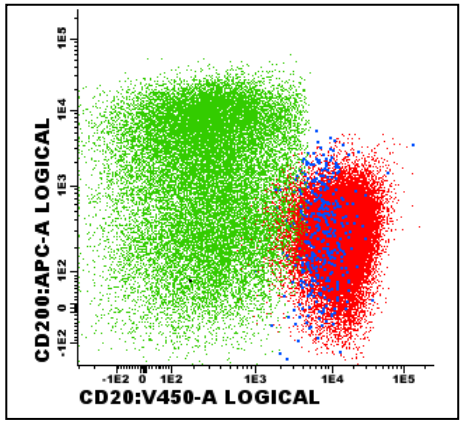
Linf. T
Linf. B patológicos

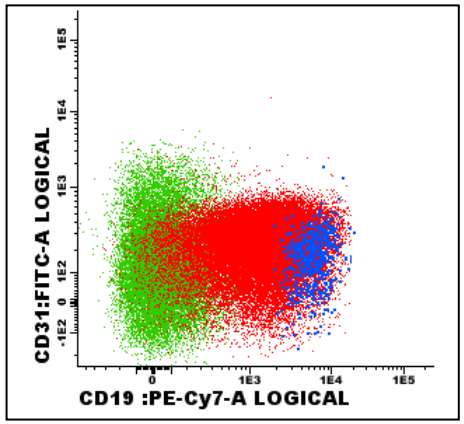
Muestra: Ganglio

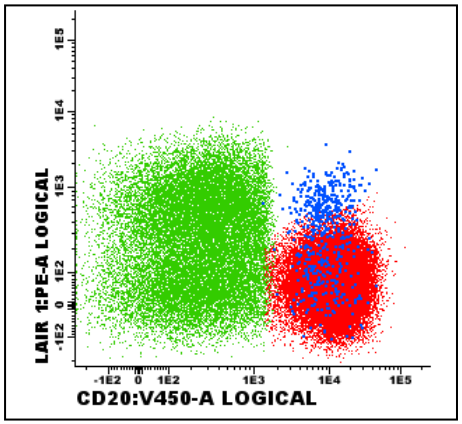


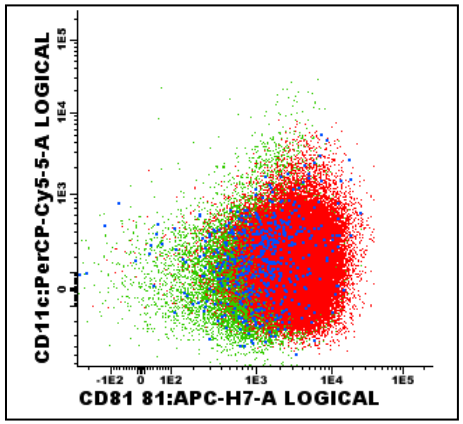


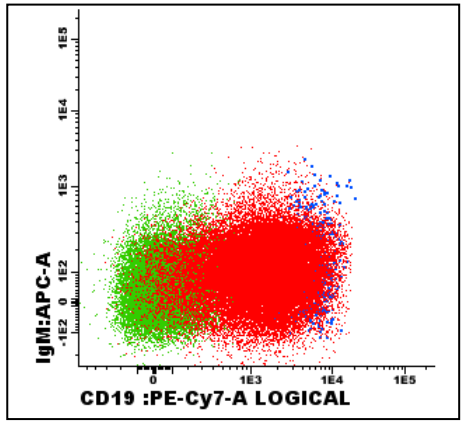








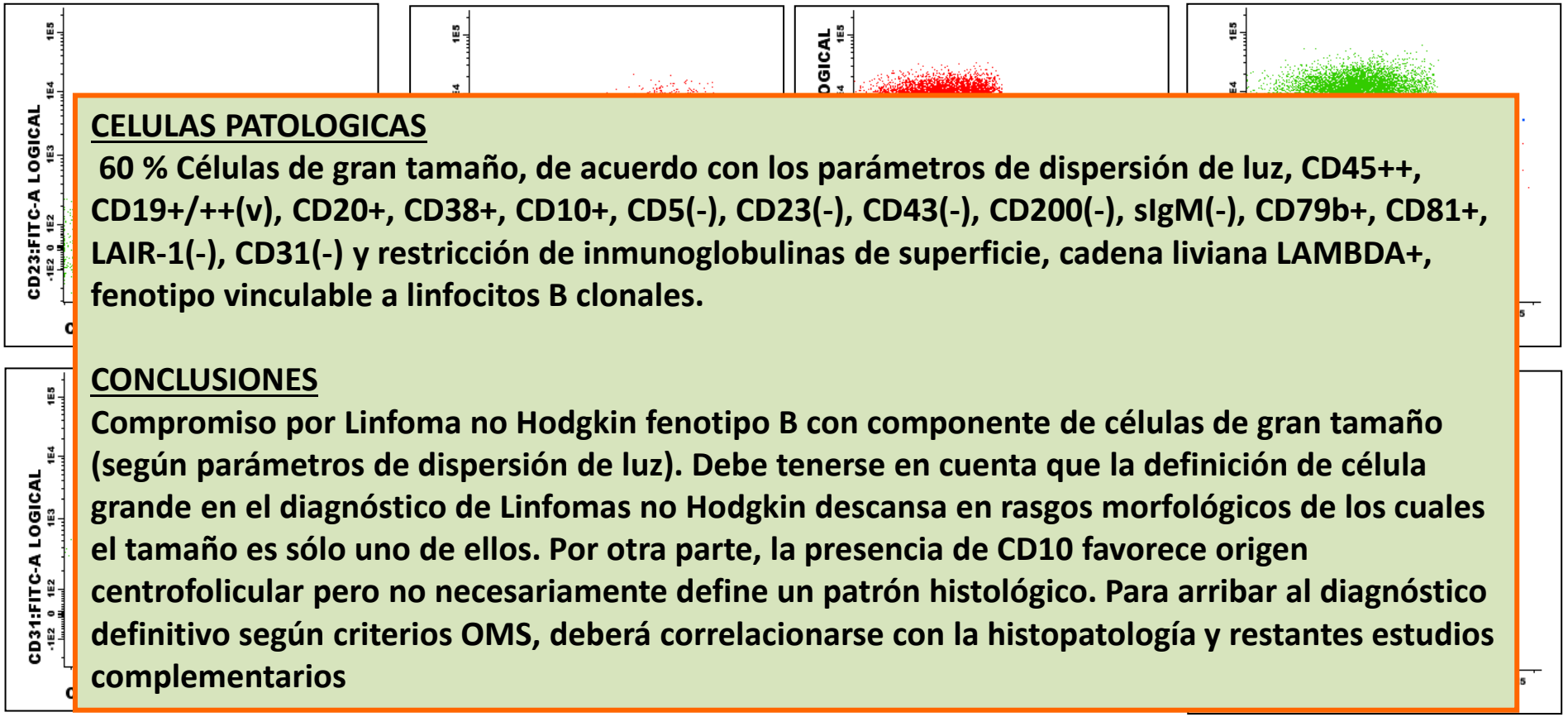




Linfoma comp. de grandes células

Linf. T
Linf. B patológicos

Muestra: Ganglio



CELULAS PATOLOGICAS

60 % Células de gran tamaño, de acuerdo con los parámetros de dispersión de luz, CD45++, CD19+/++(v), CD20+, CD38+, CD10+, CD5(-), CD23(-), CD43(-), CD200(-), sIgM(-), CD79b+, CD81+, LAIR-1(-), CD31(-) y restricción de inmunoglobulinas de superficie, cadena liviana LAMBDA+, fenotipo vinculable a linfocitos B clonales.

CONCLUSIONES

Compromiso por Linfoma no Hodgkin fenotipo B con componente de células de gran tamaño (según parámetros de dispersión de luz). Debe tenerse en cuenta que la definición de célula grande en el diagnóstico de Linfomas no Hodgkin descansa en rasgos morfológicos de los cuales el tamaño es sólo uno de ellos. Por otra parte, la presencia de CD10 favorece origen centrofolicular pero no necesariamente define un patrón histológico. Para arribar al diagnóstico definitivo según criterios OMS, deberá correlacionarse con la histopatología y restantes estudios complementarios

NOTA

La detección inmunofenotípica de neoplasias linfoides mediante citometría de flujo tiene limitaciones que incluyen la Enfermedad de Hodgkin y ciertos Linfomas No Hodgkin con componentes de grandes células.

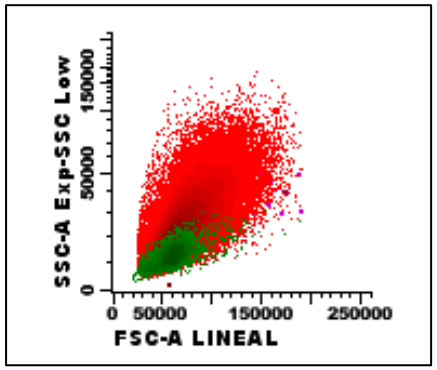
Linfoma Burkitt

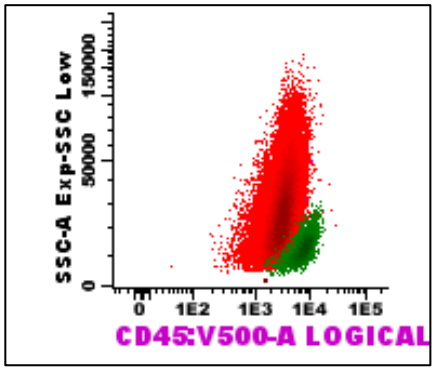
Linfocitos T Celulas NK
LINFOCITOS B CLONALES

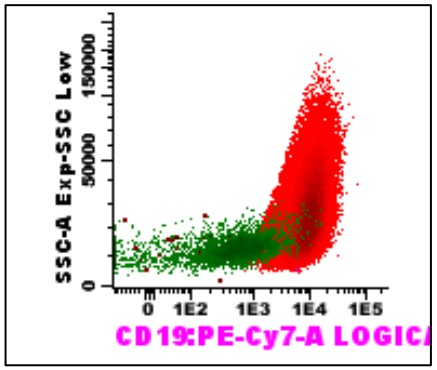
Muestra:GANGLIO

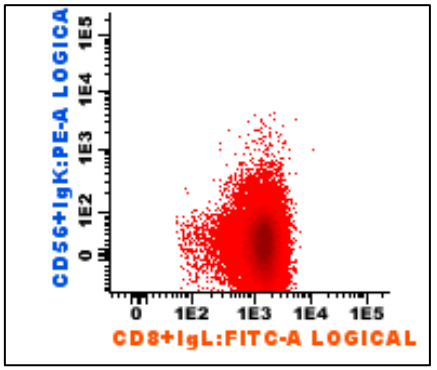
1-LST

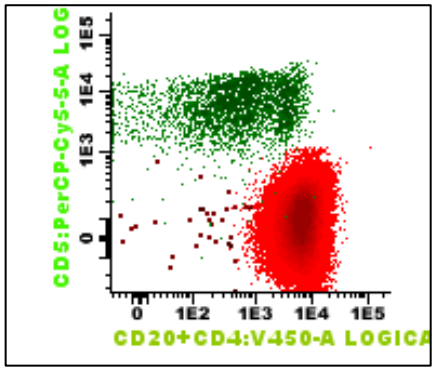
Pac Blue	Pac Orange	FITC	PE	PerCP Cy5.5	PE Cy7	APC	APC H7
CD20 CD4	CD45	Lambda CD8	Kappa CD56	CD5	CD19 TCR γδ	CD3	CD38

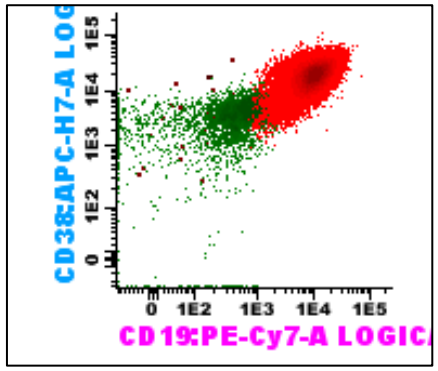


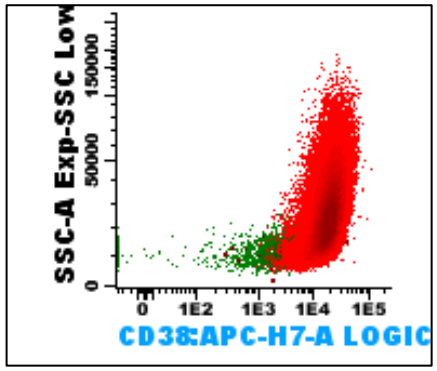




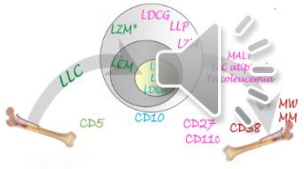








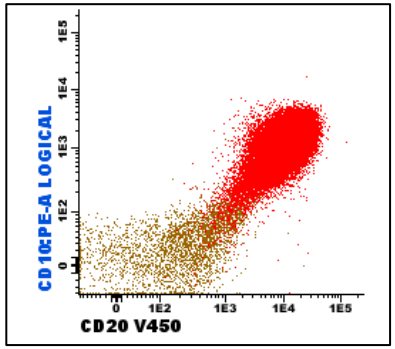
Linfoma CG

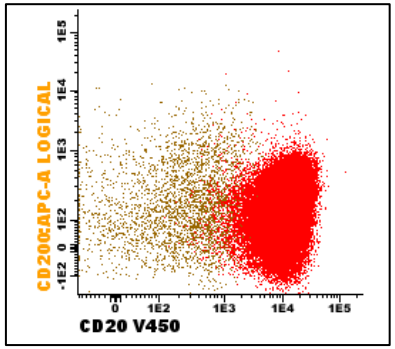


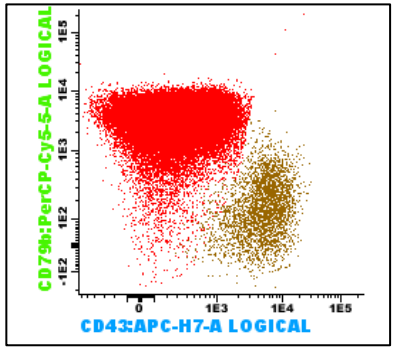
Linfoma Burkitt

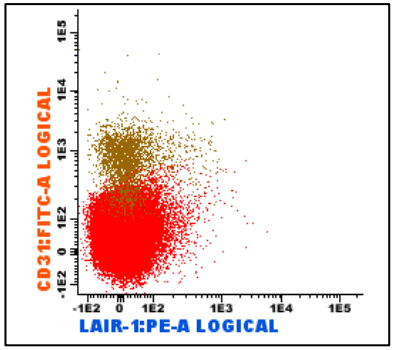
Linf. B CLONALES
Otros linfocitos

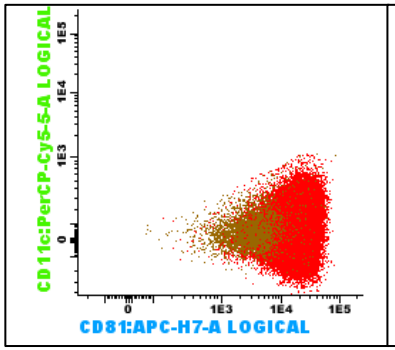
Muestra:GANGLIO

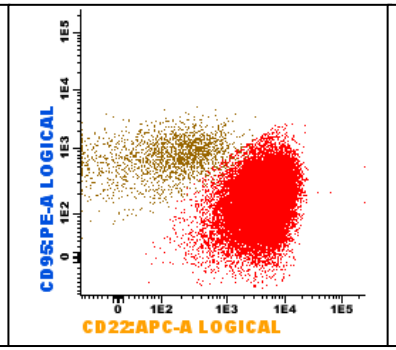


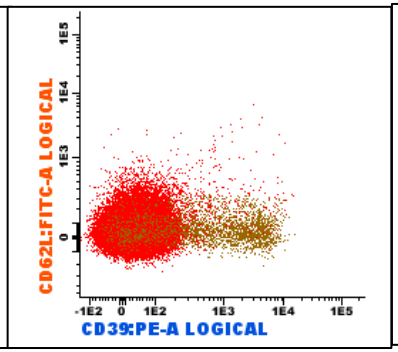


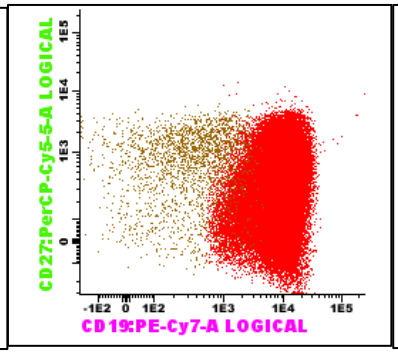


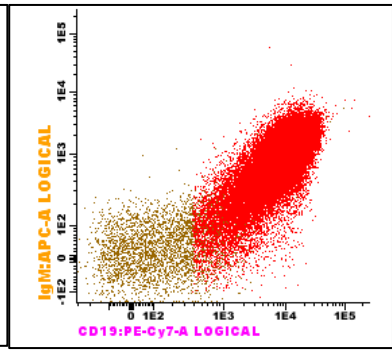




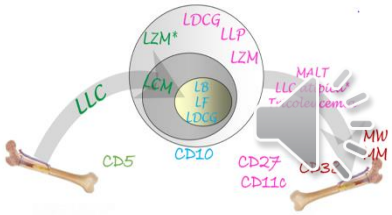








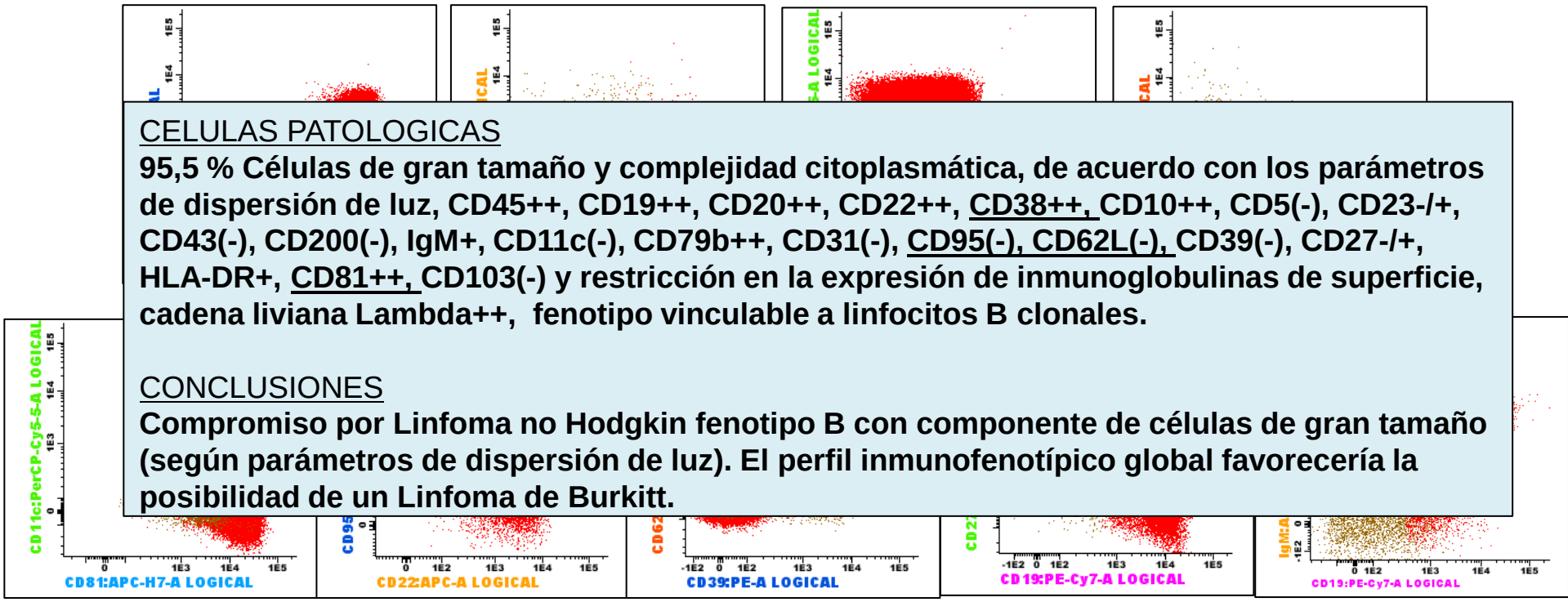
Linfoma CG



Linfoma Burkitt

Linf. B CLONALES
Otros linfocitos

Muestra:GANGLIO



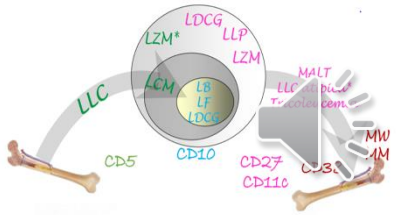
CELULAS PATOLOGICAS

95,5 % Células de gran tamaño y complejidad citoplasmática, de acuerdo con los parámetros de dispersión de luz, CD45++, CD19++, CD20++, CD22++, CD38++, CD10++, CD5(-), CD23-/+, CD43(-), CD200(-), IgM+, CD11c(-), CD79b++, CD31(-), CD95(-), CD62L(-), CD39(-), CD27-/+, HLA-DR+, CD81++, CD103(-) y restricción en la expresión de inmunoglobulinas de superficie, cadena liviana Lambda++, fenotipo vinculable a linfocitos B clonales.

CONCLUSIONES

Compromiso por Linfoma no Hodgkin fenotipo B con componente de células de gran tamaño (según parámetros de dispersión de luz). El perfil inmunofenotípico global favorecería la posibilidad de un Linfoma de Burkitt.

Linfoma CG



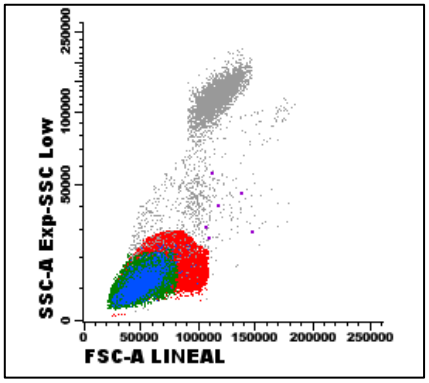
MALT/LZM/LLP

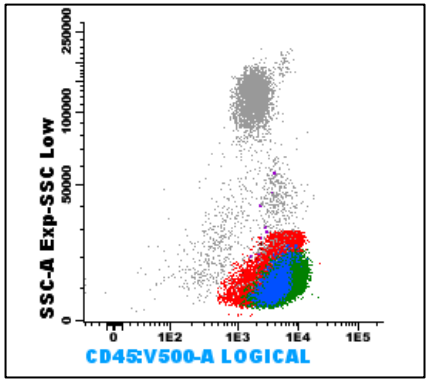
Linf. T
Linf. B policlonales
Linf. B patológicos

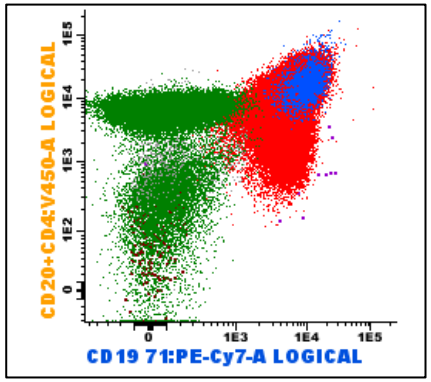
1-LST

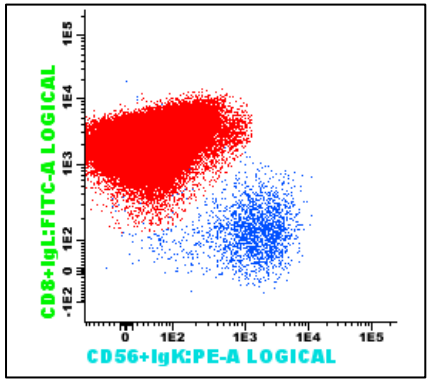
Pac Blue	Pac Orange	FITC	PE	PerCP Cy5.5	PE Cy7	APC	APC H7
CD20 CD4	CD45	Lambda CD8	Kappa CD56	CD5	CD19 TCR γδ	CD3	CD38

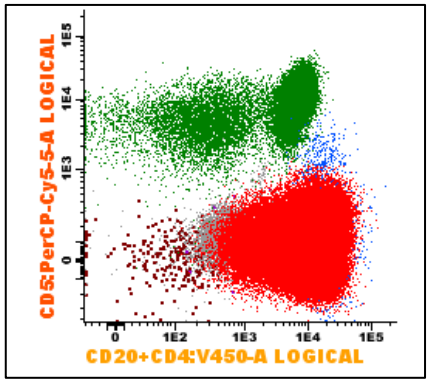
Muestra: Lesión de partes blandas

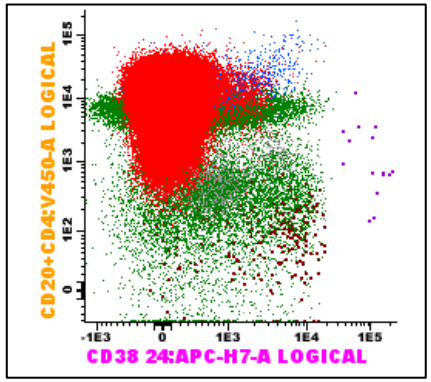




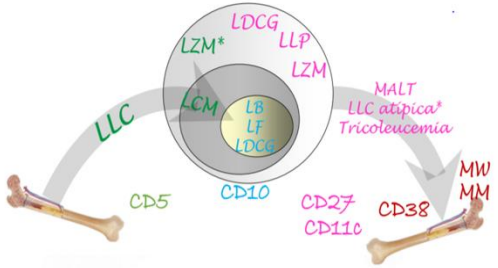








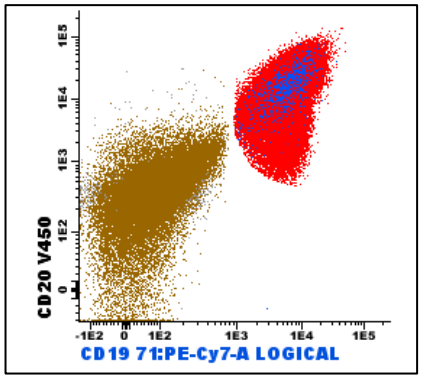
Linfoma post CG

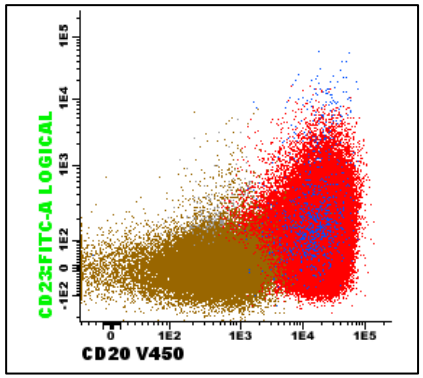


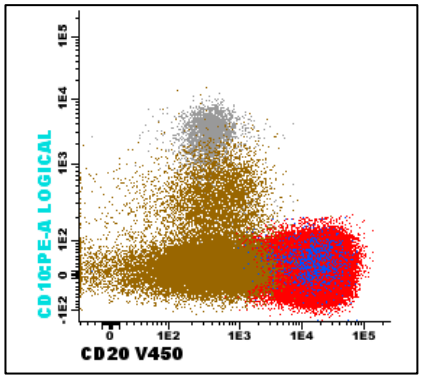
MALT/LZM/LLP

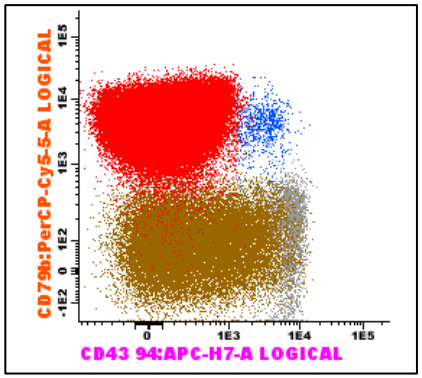
Linf. T
Linf. B policlonales
Linf. B patológicos

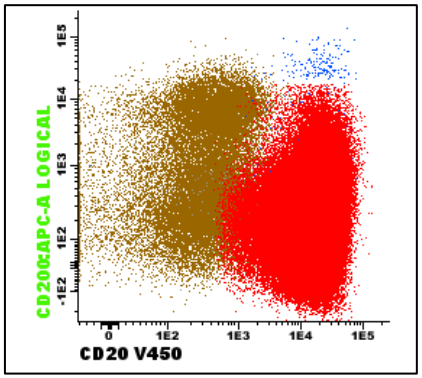
Muestra: Lesión de partes blandas

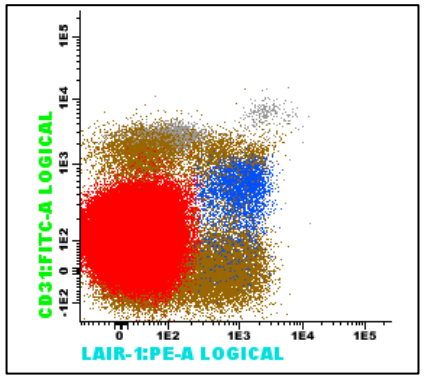


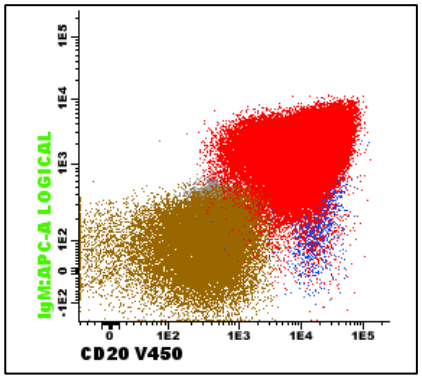


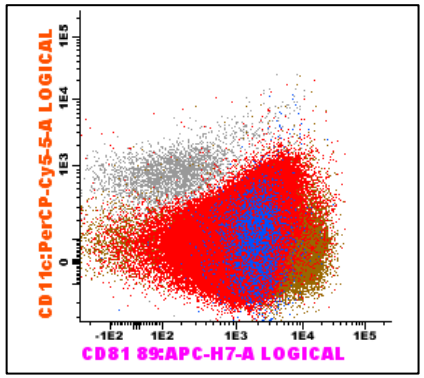




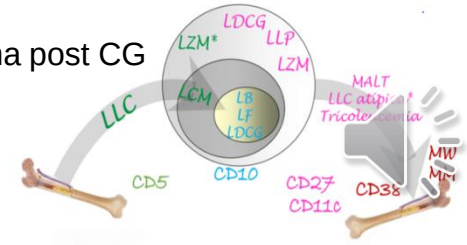








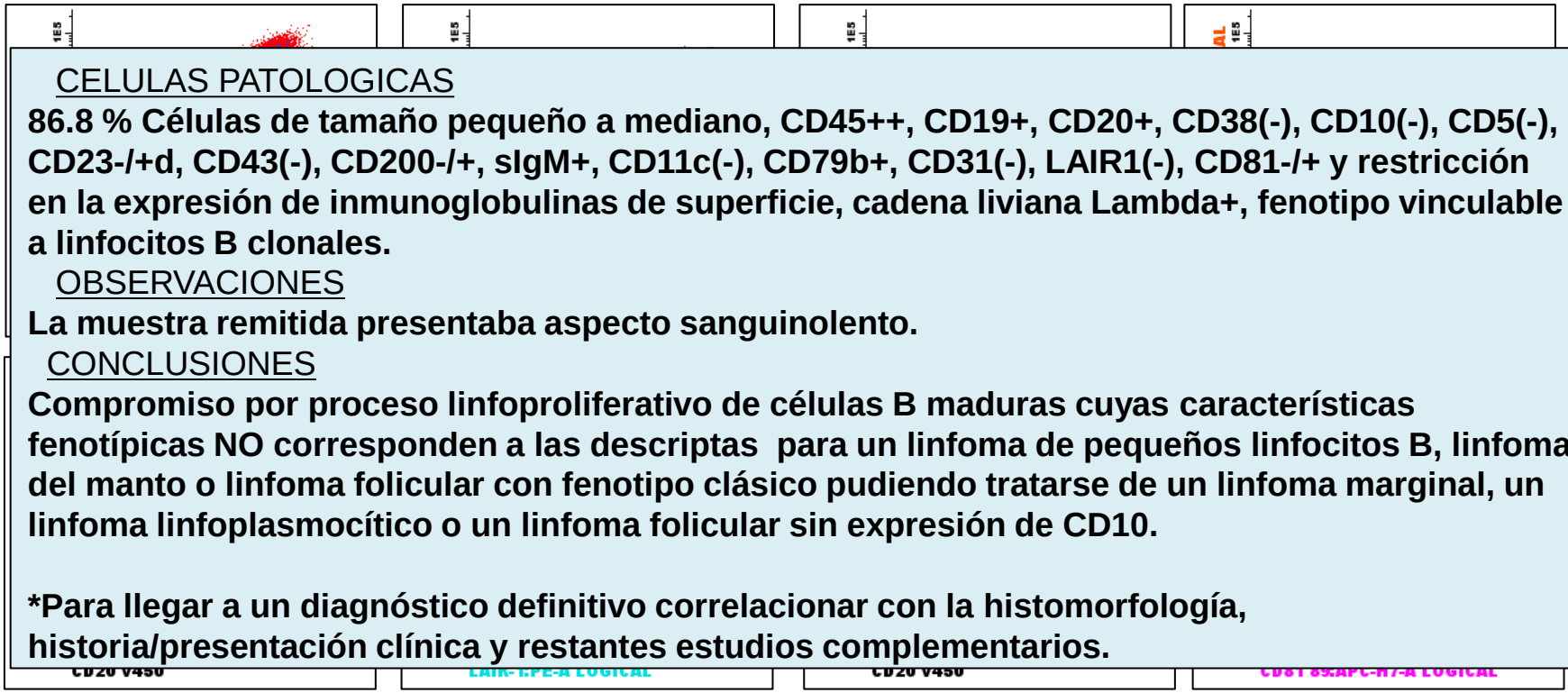
Linfoma post CG



MALT/LZM/LLP

Linf. T
Linf. B policlonales
Linf. B patológicos

Muestra: Lesión de partes blandas



CELULAS PATOLOGICAS

86.8 % Células de tamaño pequeño a mediano, CD45++, CD19+, CD20+, CD38(-), CD10(-), CD5(-), CD23-/+d, CD43(-), CD200-/+, sIgM+, CD11c(-), CD79b+, CD31(-), LAIR1(-), CD81-/+ y restricción en la expresión de inmunoglobulinas de superficie, cadena liviana Lambda+, fenotipo vinculable a linfocitos B clonales.

OBSERVACIONES

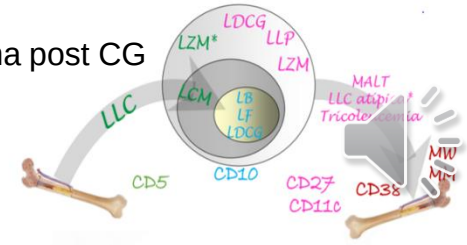
La muestra remitida presentaba aspecto sanguinolento.

CONCLUSIONES

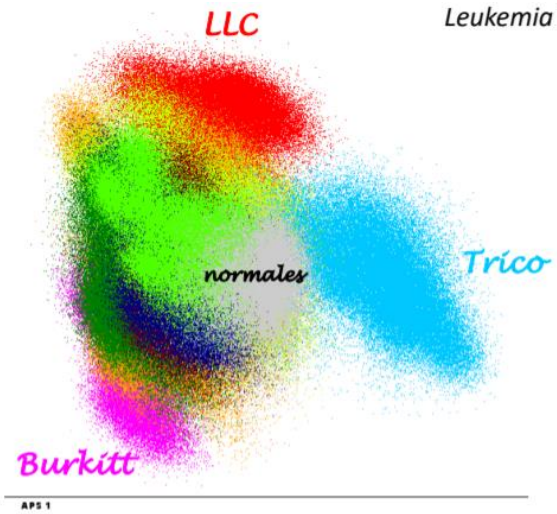
Compromiso por proceso linfoproliferativo de células B maduras cuyas características fenotípicas NO corresponden a las descriptas para un linfoma de pequeños linfocitos B, linfoma del manto o linfoma folicular con fenotipo clásico pudiendo tratarse de un linfoma marginal, un linfoma linfoplasmocítico o un linfoma folicular sin expresión de CD10.

***Para llegar a un diagnóstico definitivo correlacionar con la histomorfología, historia/presentación clínica y restantes estudios complementarios.**

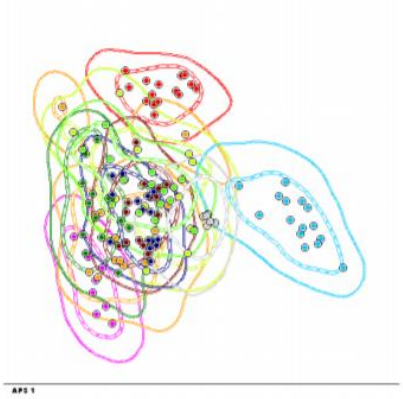
Linfoma post CG



- SLP-B son fenotípicamente diferente entre sí.



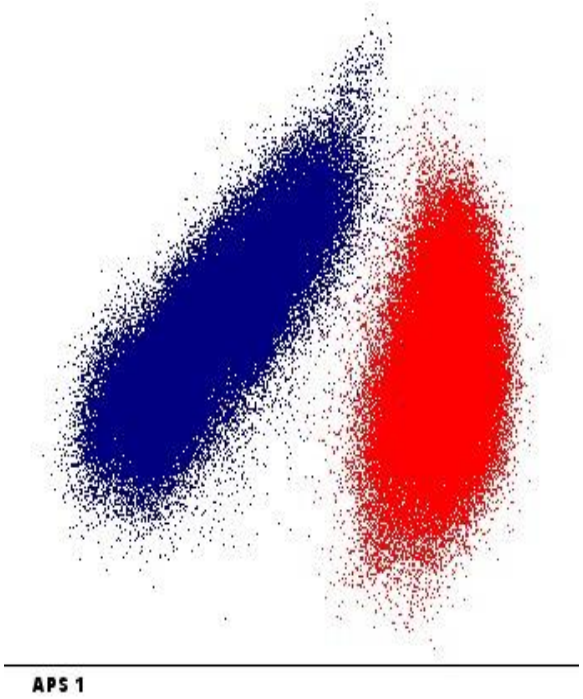
Leukemia 2012; 26, 1908–1975 (van Dongen et al on behalf of EuroFlow

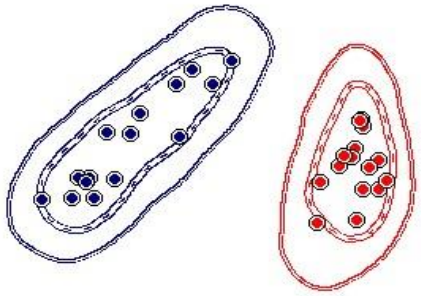


- LLC, Burkitt y Tricoleucemia presentan un fenotipo muy característico.

Utilidad de la CF para el diagnostico diferencial

LLC vs Linfoma del Manto





View 1

Parameter	Significance
PerCP Cy5-5-A:CD79b LOGICAL	13.15
APC-A:CD200 LOGICAL	12.88
APC-A:IgM LOGICAL	11.96
FITC-A:CD23 LOGICAL	7.16
PB-A:CD20 LOGICAL	7.06
APC H7-A:CD43 LOGICAL	6.13
PE-A:LAIR1 LOGICAL	4.60
PerCP Cy5-5-A:CD22 LOGICAL	4.26
FITC-A:CD62L LOGICAL	3.27
APC H7-A:CD38 LOGICAL	3.25

View 2

Parameter	Significance
APC H7-A:CD43 LOGICAL	8.10
APC-A:CD27 LOGICAL	7.92
FITC-A:CD23 LOGICAL	7.77
PE-A:CD56+IgK LOGICAL	6.97
APC-A:CD200 LOGICAL	6.70
PerCP Cy5-5-A:CD5 LOGICAL	6.01
PE-A:CD39 LOGICAL	5.19
FITC-A:CD62L LOGICAL	4.67
APC-A:CXCR5 LOGICAL	3.95
FITC-A:CD31 LOGICAL	3.78
APC H7-A:CD49d LOGICAL	3.32

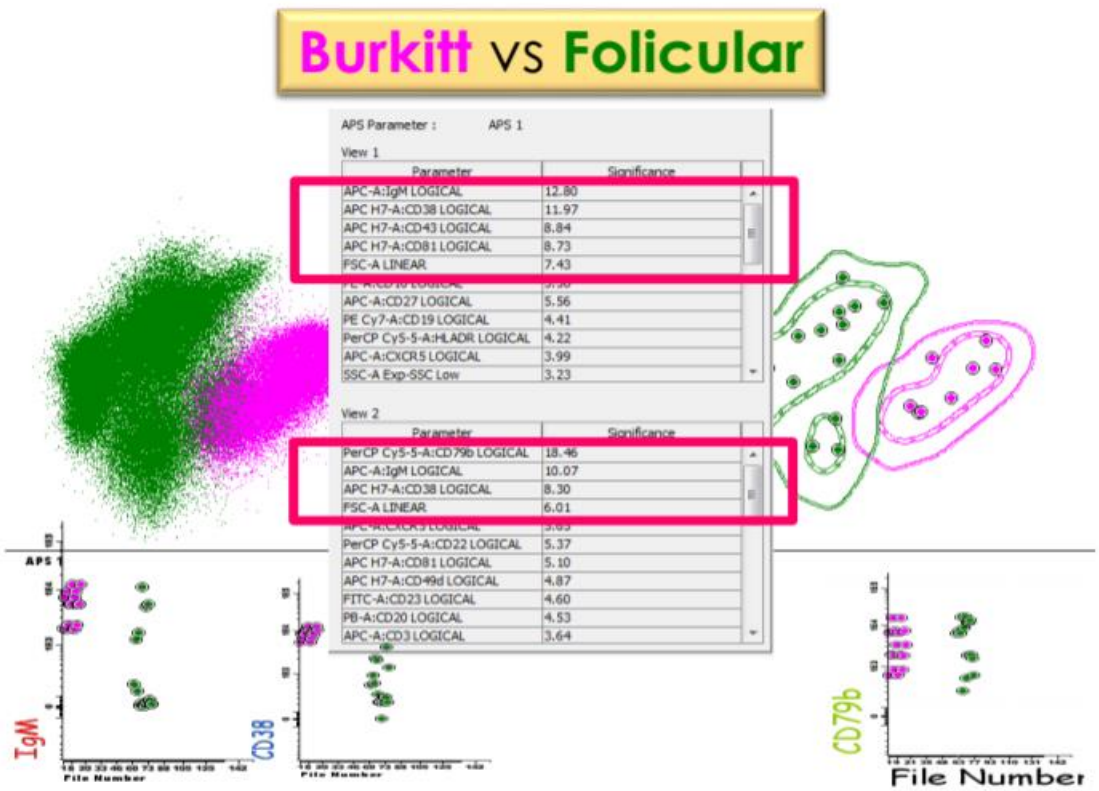
Importancia:

CD79b 13,15

CD200 12,88

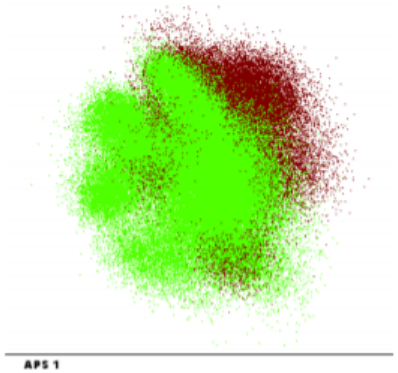
IgM 11,96

Utilidad de la CF para el diagnostico diferencial



Ig M
CD38
CD79b

LZM-LLP-MALT



Algunos SLP-B tienen PATRONES FENOTIPICOS MUY HETEROGENEOS

Algoritmo diagnóstico para SLPC-B

Célula pequeña (FSC < FSC Linf T)

CD19+ Hom
CD20+ Hom

NO LLC *CD20+d*
NO LF *CD19+d*

CD5

- MALT / LLP / LZM

+ MALT / LLP / LZM VS MANTO

Célula grande (FSC > FSC Linf T)

CD19+ Hom
CD20+ Hom

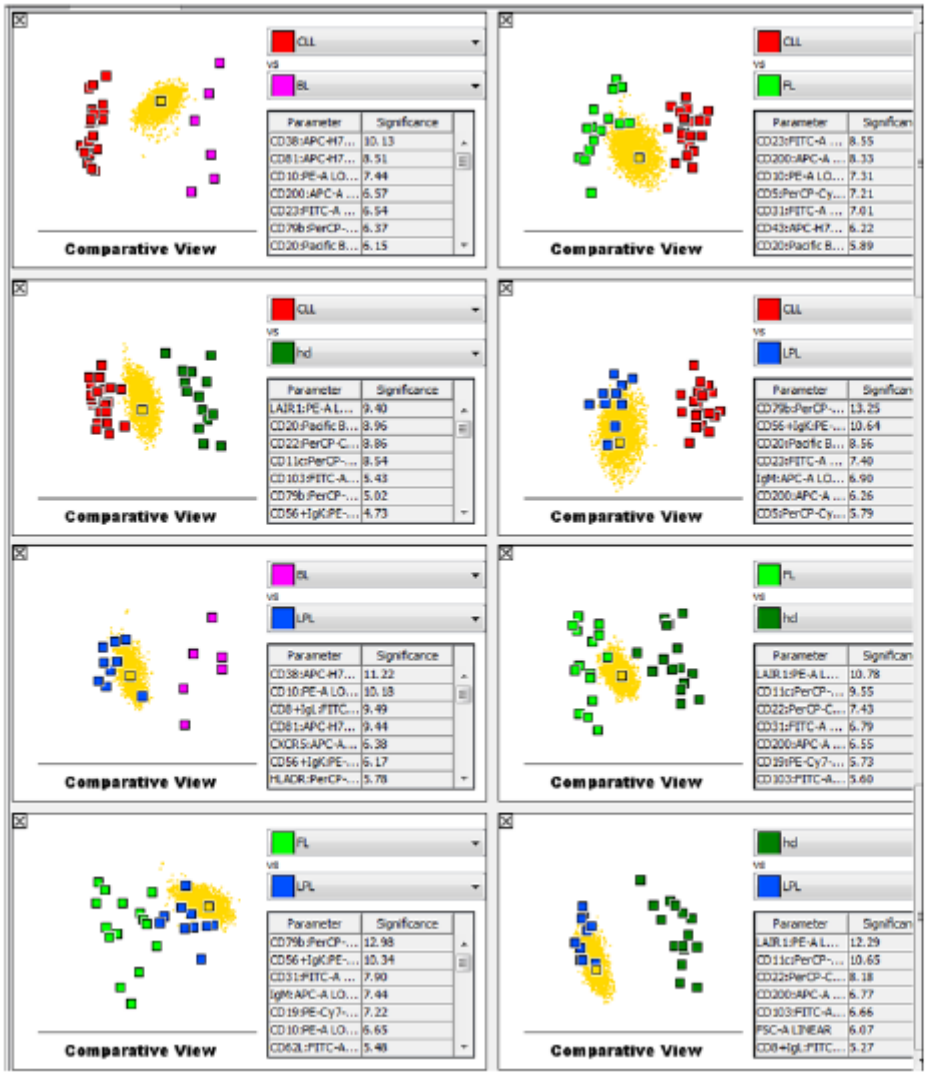
NO TRICO *CD19+++ & CD20+++*

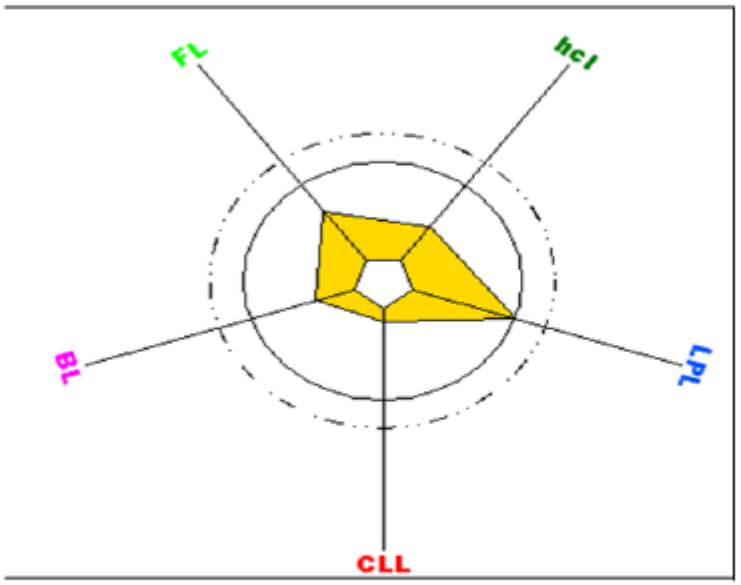
CD10

- LCG CD10-

+ LCG CD10+ VS BURKITT

Análisis comparativo de un caso vs varios grupos de referencia





Responsible scientists: Sebastian Buttcher

Linfocitosis B monoclonal (MBL)

- ✓ **Presencia de un clon de células B con un recuento absoluto < 5000 cél/ul sin otros signos de desorden linfoproliferativo (ausencia de citopenias, linfoadenopatias, hepatomegalia o esplenomegalia).**

- ✓**La mayoría presentan fenotipo tipo LLC-B.**
- ✓**La prevalencia es del 3,5% a 12% en adultos mayores de 40 años.**

- ✓ **Se clasifican:** ***MBL de bajo recuento: < 500/ul**
  ***MBL de alto recuento: > 500/ul**

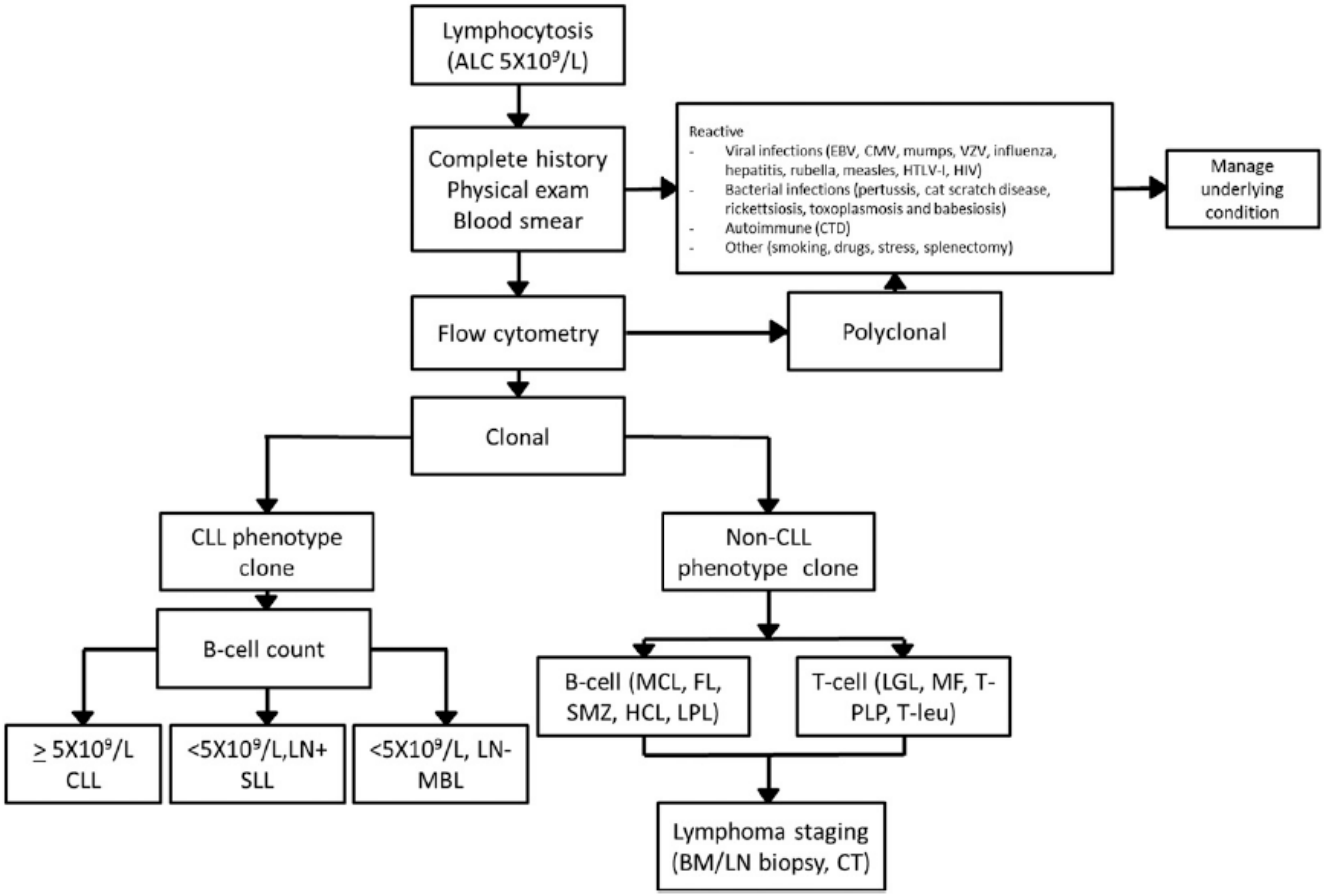
MBL Bajo recuento

- ✓ se detectan en aprox. 5% de adultos mayores de 40 años con estudios de *alta sensibilidad* (CMF).
- ✓ Poco frecuente progresión a LNH-B.

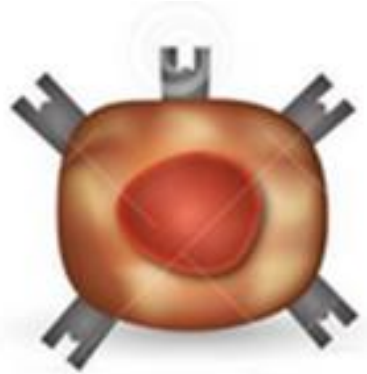
MBL alto recuento

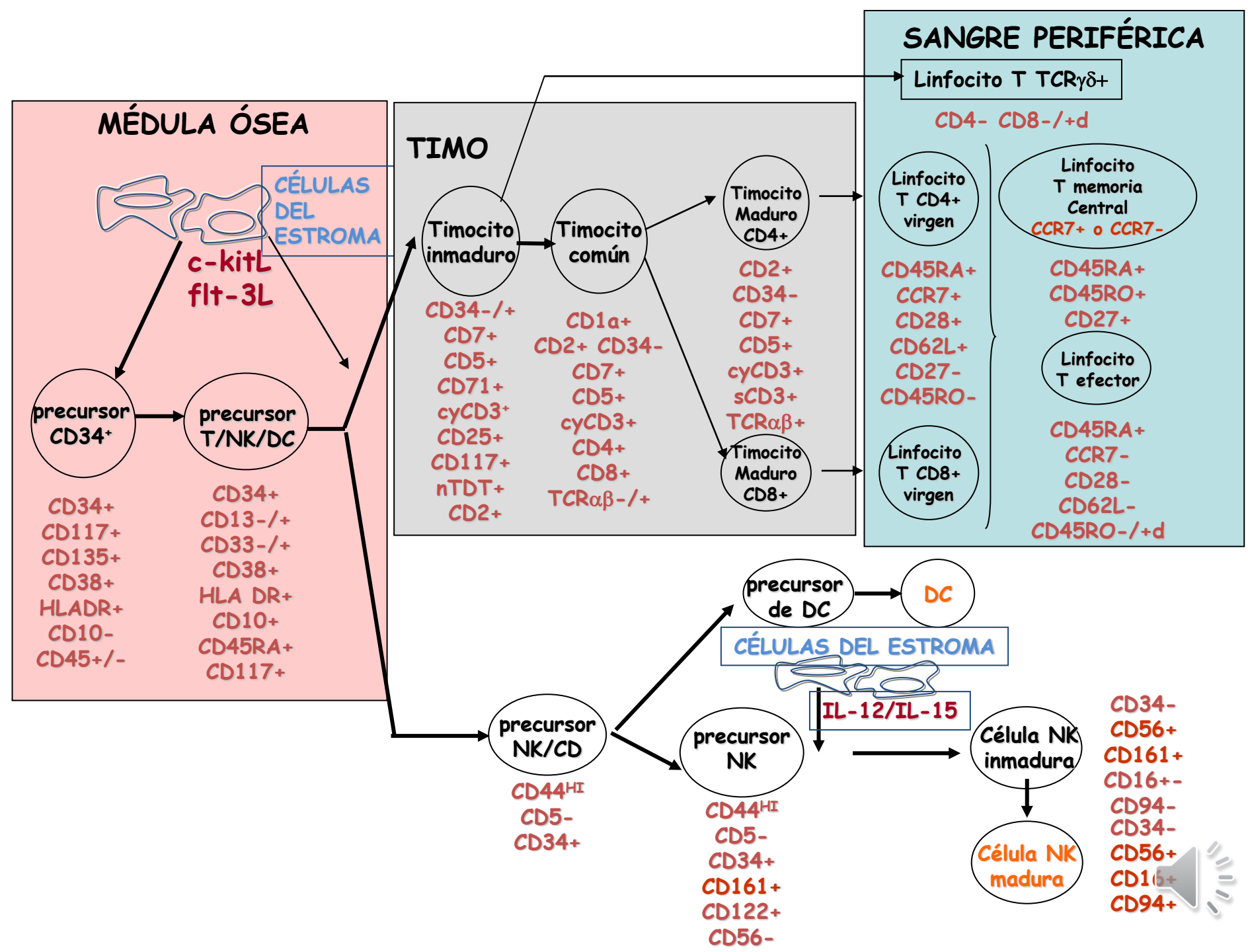
- ✓ Progresan a LNH-B requiriendo tratamiento entre 1-2% por año.

Linfocitosis B monoclonal (MBL)

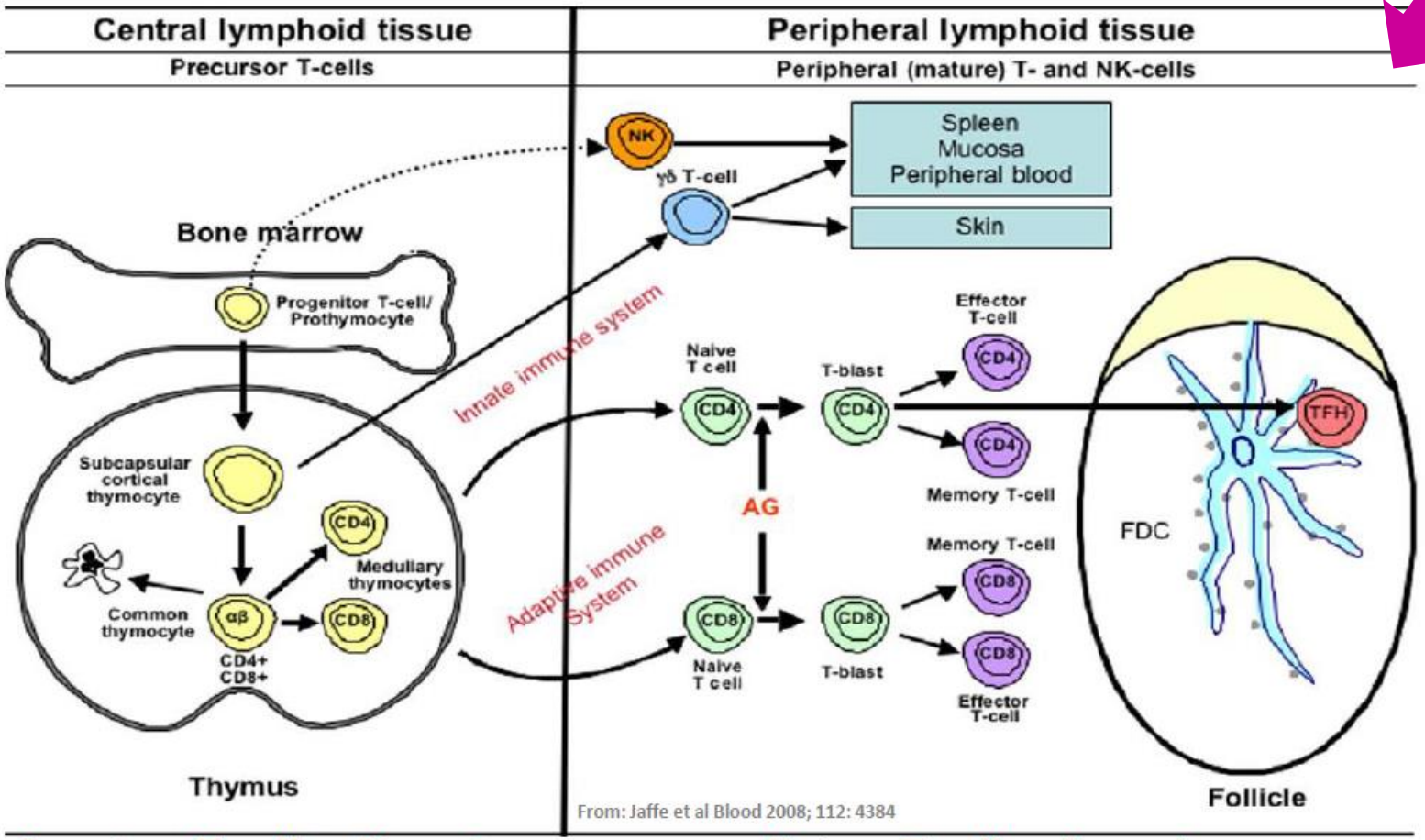


LINFOCITOS T




MÉDULA ÓSEA
CÉLULAS DEL ESTROMA
c-kitL
flt-3L
precursor CD34+
CD34+ CD117+ CD135+ CD38+ HLADR+ CD10- CD45+/-
precursor T/NK/DC
CD34+ CD13-/+ CD33-/+ CD38+ HLA DR+ CD10+ CD45RA+ CD117+
TIMO
Timocito inmaduro
CD34-/+ CD7+ CD5+ CD71+ cyCD3+ CD25+ CD117+ nTDT+ CD2+
Timocito común
CD1a+ CD2+ CD34- CD7+ CD5+ cyCD3+ CD4+ CD8+ TCRαβ-/+
Timocito Maduro CD4+
CD2+ CD34- CD7+ CD5+ cyCD3+ sCD3+ TCRαβ+
Timocito Maduro CD8+
SANGRE PERIFÉRICA
Linfocito T TCRγδ+
CD4- CD8-/+d
Linfocito T CD4+ virgen
CD45RA+ CCR7+ CD28+ CD62L+ CD27- CD45RO-
Linfocito T memoria Central
CCR7+ o CCR7-
CD45RA+ CD45RO+ CD27+
Linfocito T efector
CD45RA+ CCR7- CD28- CD62L- CD45RO-/+d
Linfocito T CD8+ virgen
precursor NK/CD
CD44HI CD5- CD34+
precursor de DC
DC
CÉLULAS DEL ESTROMA
IL-12/IL-15
precursor NK
CD44HI CD5- CD34+ CD161+ CD122+ CD56-
Célula NK inmadura
CD34- CD56+ CD161+ CD16+- CD94-
Célula NK madura
CD34- CD56+ CD16+ CD94+

Neoplasias de células T



Immature T-cell malignancies (thymic origin) 15-20%:

-T-cell acute lymphoblastic leukemias
-TdT+ T-cell lymphoblastic lymphomas

Mature T-cell malignancies (post-thymic origin) 80-85%:

-Peripheral T-cell leukemias
-Peripheral T-cell lymphomas
- Vast majority TCRαβ+
- TCRγδ+ are rare

Neoplasias de células T

Leucemia

SP y MO

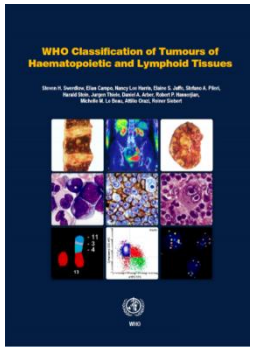
Linfoma

Nodal

Ganglios linfáticos

Extranodal

Piel
Bazo
Hígado
TGI
Tracto respiratorio



Neoplasias de células T y NK maduras

Primarily leukemic/disseminated:

- T-cell prolymphocytic leukemia
- T-cell LGL leukemia
- Chronic LPD of NK cells*
- Aggressive NK-cell leukemia
- Adult T-cell lymphoma/leukemia (HTLV1+)
- Systemic EBV+ T-cell LPD of childhood

Nodal:

- Angioimmunoblastic T-cell lymphoma
- Anaplastic large cell lymphoma, *ALK+*
- Anaplastic large cell lymphoma, *ALK-* *
- Peripheral T-cell lymphoma not otherwise specified (NOS)

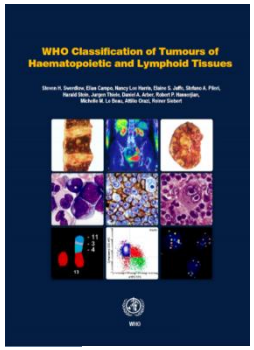
Extranodal:

- Extranodal NK/T-cell lymphoma, nasal type
- Enteropathy associated T-cell lymphoma
- Hepatosplenic T-cell lymphoma
- Subcutaneous panniculitis-like T-cell lymphoma

- T Sézary syndrome / Mycosis fungoides
- Primary cutaneous CD30+ T-cell LPD
  - Primary cutaneous anaplastic large cell lymphoma
  - Lymphomatoid papulosis
- Primary cutaneous gamma-delta T-cell lymphoma
- Primary cutaneous CD8+ agressive epidermotropic cytotoxic T-cell lymphoma*
- Primary cutaneous CD4+ small/medium T-cell lymphoma*

Predominant cutaneous presentation

* Provisional entities



Neoplasias de células T y NK maduras

Primarily leukemic/disseminated:

- T-cell prolymphocytic leukemia (*TCL1* gen)
- T-cell LGL leukemia (activating STAT3/5b mut)
- Chronic LPD of NK cells*
- Aggressive NK-cell leukemia
- Adult T-cell lymphoma/leukemia (HTLV1+)
- Systemic EBV+ T-cell LPD of childhood

Nodal:

- Angioimmunoblastic T-cell lymphoma (*IDH2* mut)
- Anaplastic large cell lymphoma, *ALK*+ (t(2,5))
- Anaplastic large cell lymphoma, *ALK*- * (t(6,7))**
- Peripheral T-cell lymphoma not otherwise specified (NOS)

Extranodal:

- Extranodal NK/T-cell lymphoma, nasal type
- Enteropathy associated T-cell lymphoma
- Hepatosplenic T-cell lymphoma (iso7q, often associated with trisomy 13)
- Subcutaneous panniculitis-like T-cell lymphoma

- T Sézary syndrome / Mycosis fungoides
- Primary cutaneous CD30+ T-cell LPD
  - Primary cutaneous anaplastic large cell lymphoma
  - Lymphomatoid papulosis
- Primary cutaneous gamma-delta T-cell lymphoma
- Primary cutaneous CD8+ agressive epidermotropic cytotoxic T-cell lymphoma*
- Primary cutaneous CD4+ small/medium T-cell lymphoma*

Predominant cutaneous presentation

* Provisional entities ** Needs confirmation

Neoplasias de células T

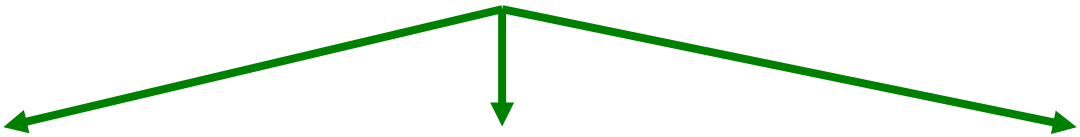
- Baja incidencia

5-10% de los SLP en Occidente, 15-20% en Asia

- Heterogeneidad clínico-biológica
- Falta de marcadores genéticos característicos
- Evaluación de clonalidad más compleja que en los SPL-B

Es imprescindible conocer el inmunofenotipo normal de las células T

Estrategias para el estudio fenotípico de las **neoplasias de células T**



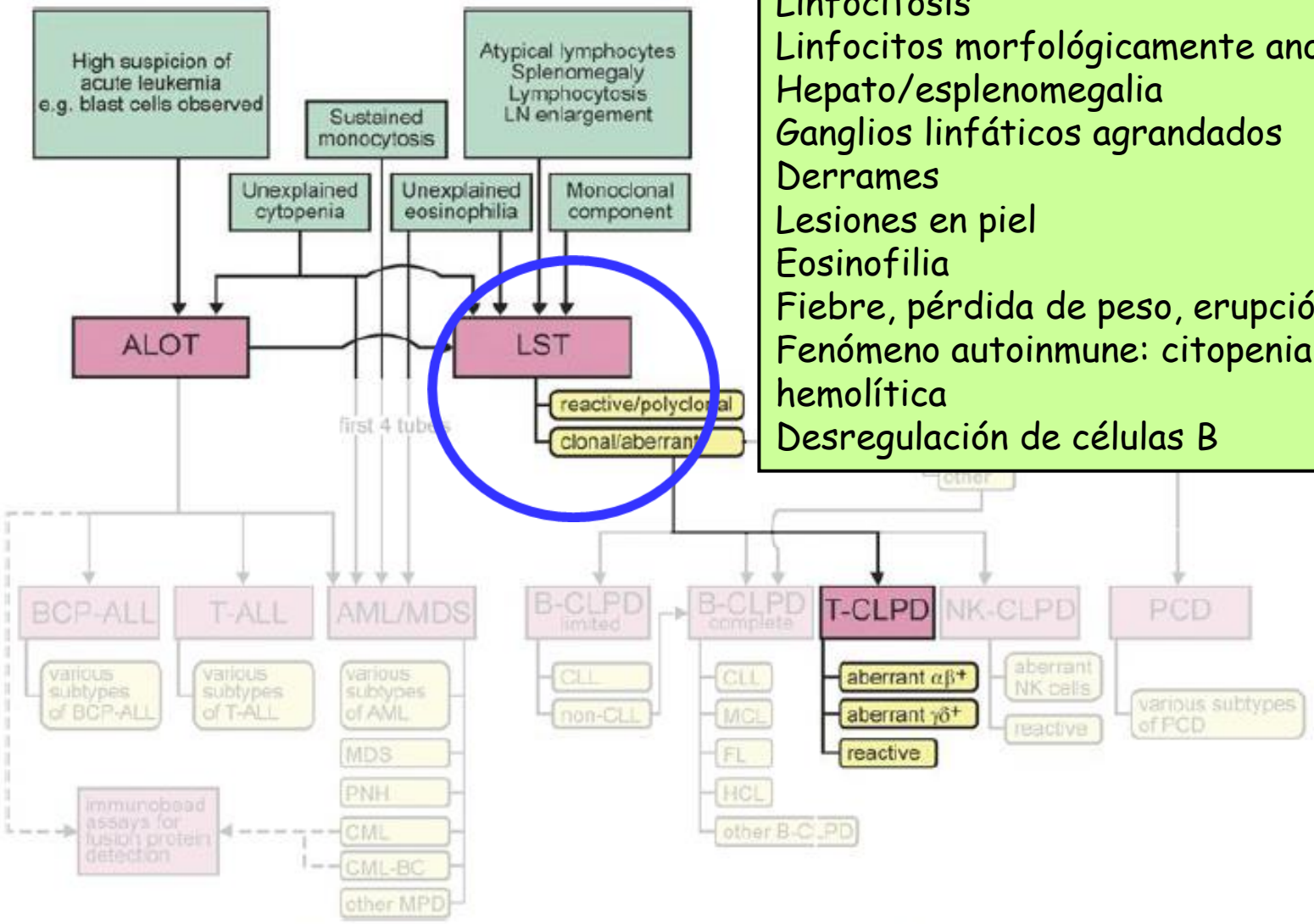
Identificar Cuantificar Caracterizar

- ✓ Tipo de célula (CD4+, CD8+, CD4+CD8+, CD4-CD8-, TCRαβ, TCRγδ)
- ✓ Establecer criterios de clonalidad: fenotipo aberrante, uso de TCRVβ
- ✓ Estado de maduración (naive, memoria, efector)
- ✓ Propiedades funcionales (expresión de Ags asociados a cél. NK/citotoxicidad)
- ✓ Estado de activación (reposo/activada)
- ✓ Tipo de muestra/clínica →localización (leucémico, nodal, extranodal)

Antígenos evaluados en neoplasias de células T

- «pan T-cell»: CD3, CD4, CD8, CD7, CD2, CD5, TCR$\alpha\beta$, TCR$\gamma\delta$ and CD45
- antígenos co-estimuladores: CD28, CD27, CD26
- relacionados a maduración: CD45RA, CD45RO
- receptores de migración (homing): CCR7 (CD197)
- Relacionado a activación celular: CD38, HLA-DR, CD25, CD11b, CD11c
- asociado a células NK: CD16, CD94
- Relacionado a citotoxicidad: CD56, CD57, Granzyme, Perforin
- Marcadores de clasificación: CD30 (ALCL), CD10 (AITL), cyTCL1 (PLL), CD279/PD-1 (AITL)

  -> expresión alterada (up/downregulation)
  -> patrones anormales



Linfocitosis
Linfocitos morfológicamente anormales
Hepato/esplenomegalia
Ganglios linfáticos agrandados
Derrames
Lesiones en piel
Eosinofilia
Fiebre, pérdida de peso, erupción dérmica
Fenómeno autoinmune: citopenia, anemia hemolítica
Desregulación de células B

Leukemia 2012; 26, 1908–1975 (van Dongen et al on behalf of EuroFlow)

EuroFlow

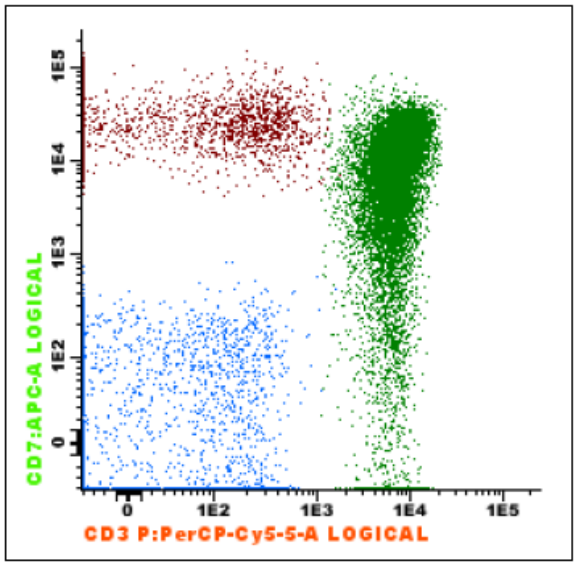
LST(Tubo de Screening Linfoide)

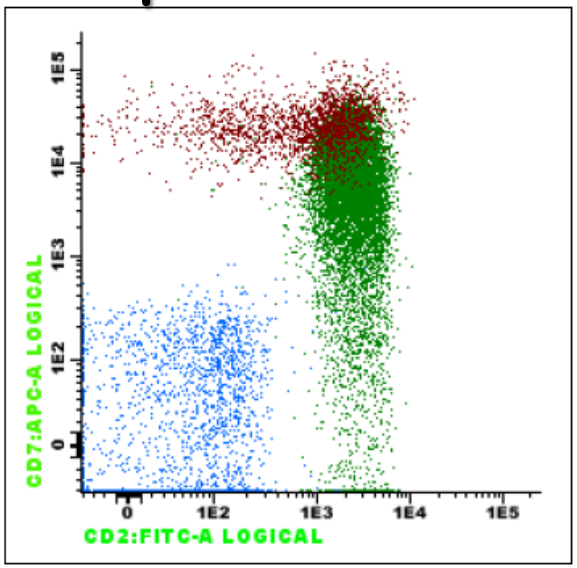
Pac Blue	Pac Orange	FITC	PE	PerCP Cy5.5	PE Cy7	APC	APC H7
CD20 CD4	CD45	Lambda CD8	Kappa CD56	CD5	CD19 TCR γδ	CD3	CD38

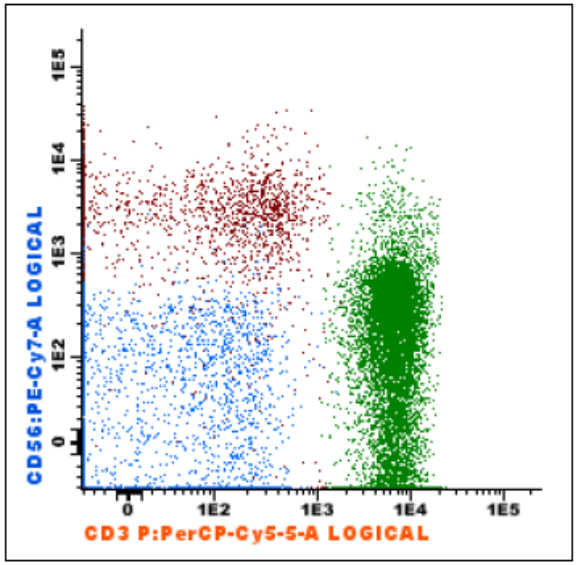
Linfocitos T

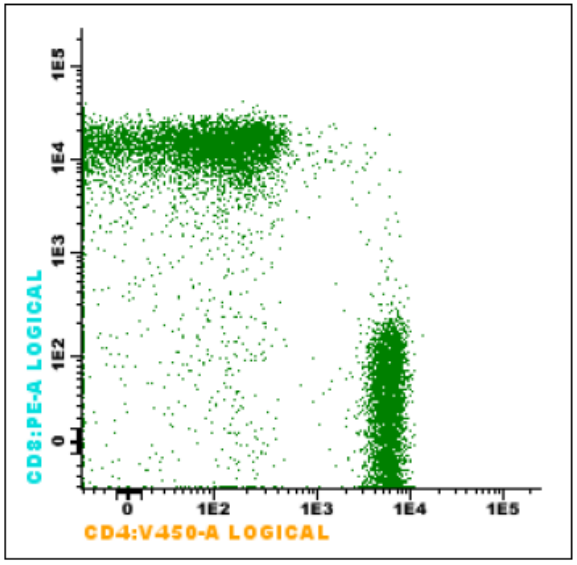
*Identificar células T y los sub-sets de células T: CD3, CD4 y CD8. TCRgd. Aberraciones T más frecuente: CD5.

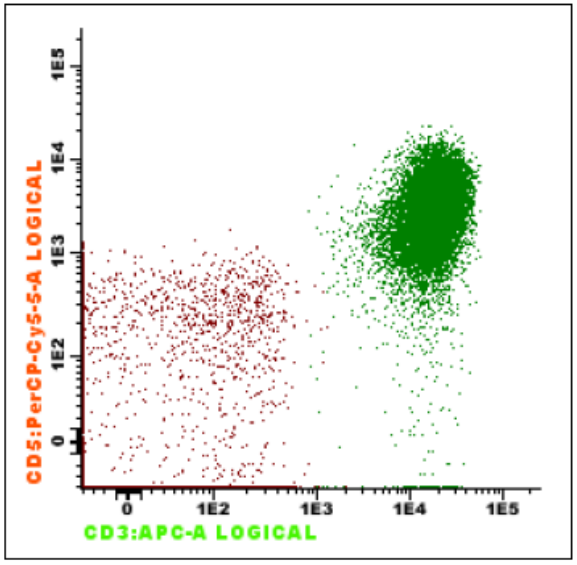
Patrón fenotípico de LT normales



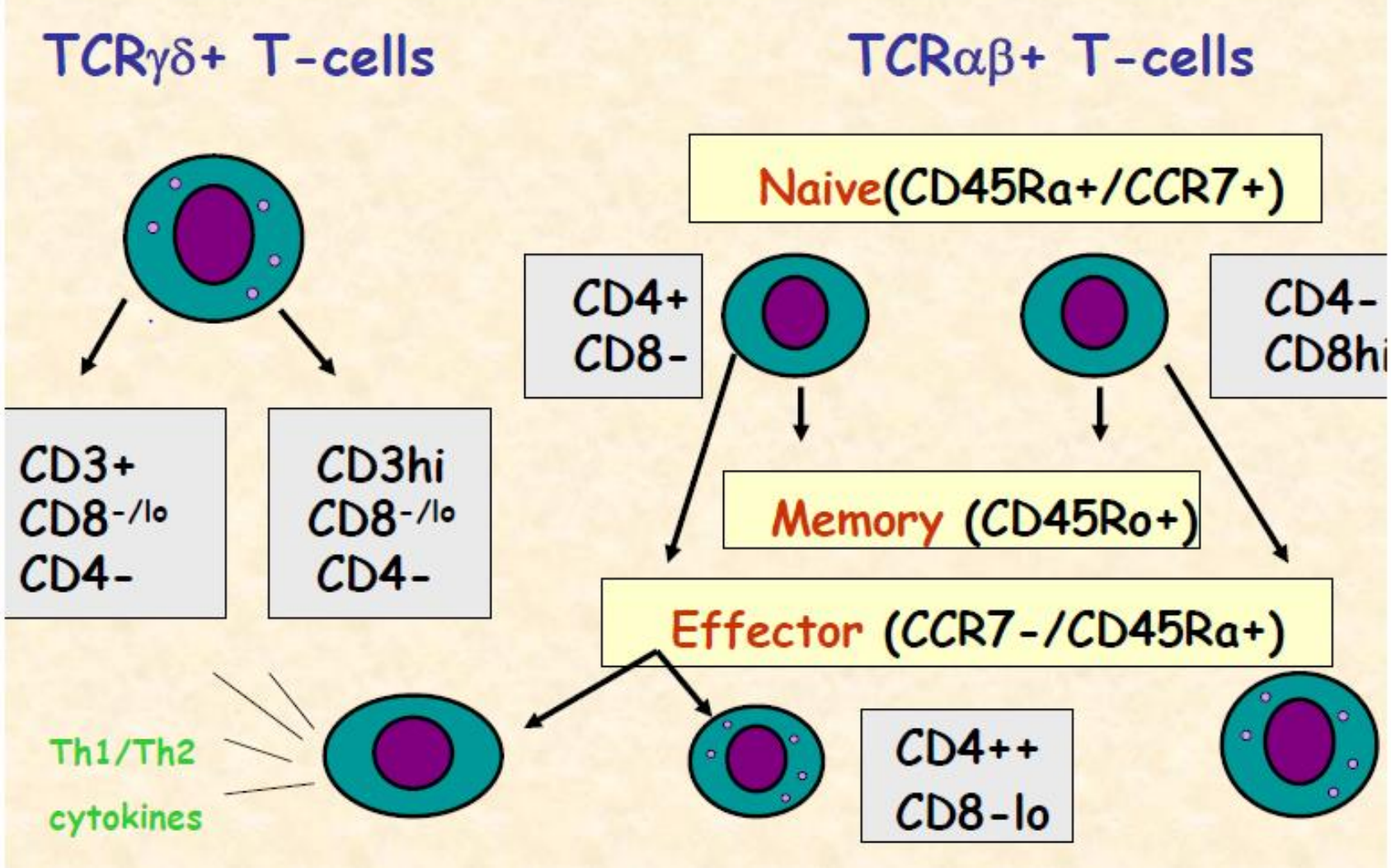








NORMAL T-CELL SUBSETS



Sospecha de neoplasia de células T maduras

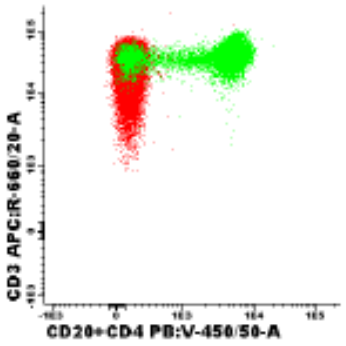
LST

Expresión de Ags alterada

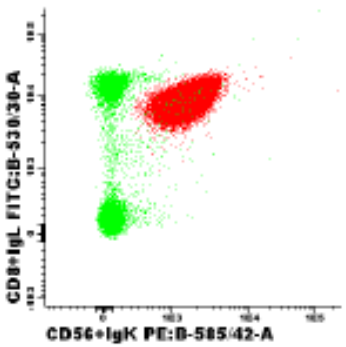
Alteraciones del scatter

Relación CD4/CD8 alterada
(> 4 ó < 0,7)

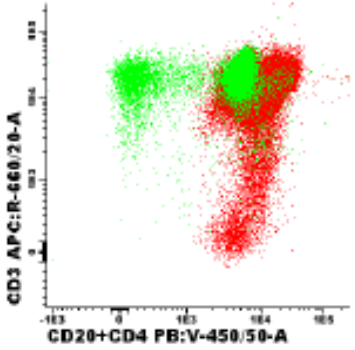
CD3+(dim)



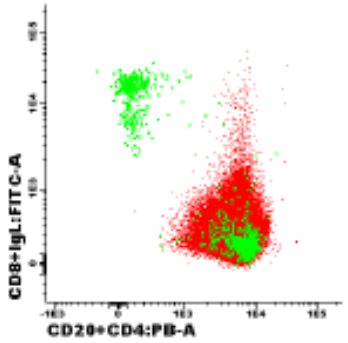
CD8+(dim)CD56+
CD4/CD8 ratio 0.02



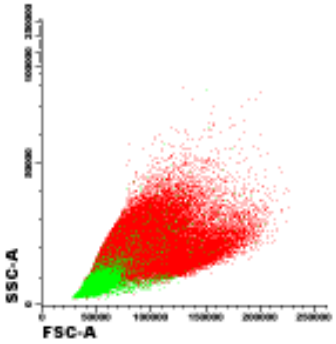
CD3+/-, CD4++



CD4/CD8 ratio: 960



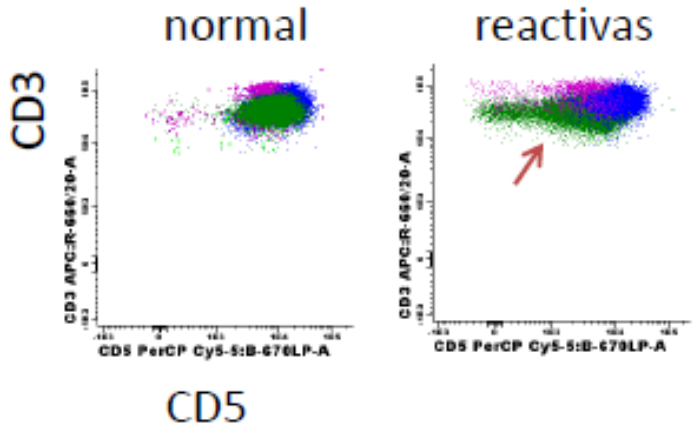
scatter

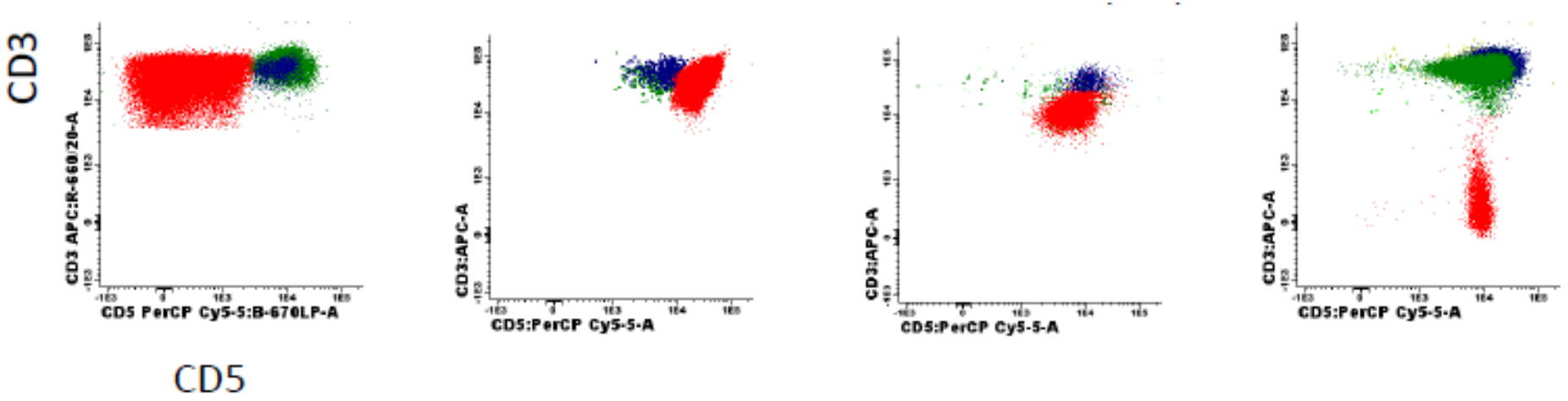


Sospecha de neoplasia de células T maduras

LST

Expresión de Ags alterada





Panel para SLP-T

	Pac.B	Pac.O	FITC	PE	PerCP-Cy5.5	PECY7	APC	APCH 7
Step 1 (screening tube)	**CD4** CD20	**CD45**	**CD8** Anti-sIgλ	**CD56** Anti-sIgκ	**CD5**	**Anti-TCRγδ** CD20	**SmCD3**	CD38
Step 2 (T-cell clonality study)	CD4	-	TCR-Vβ"A" + TCR-Vβ"B"	TCR-Vβ"B" + TCR-Vβ"C"	CD3	-	CD8	-
Step 3 (T-cell CLPD panel)	CD4	CD45	CD7	CD26	CD3	CD2	CD28	CD8
	CD4	CD45	CD27	CD197	CD3	CD45RO	CD45RA	CD8
	CD4	CD45	CD5	CD25	CD3	HLADR	cytTCL1	CD8
	CD4	CD45	CD57	CD30	CD3	-	CD11c	CD8
	CD4	CD45	cytPERF	cytGRZ	CD3	CD16	CD94	CD8

cytPERF: cytoplasmic perforin; cytGRZ: cytoplasmic granzyme B

Leukemia 2012; 26, 1908-1975 (van Dongen et al on behalf of EuroFlow)

EuroFlow

Cómo determinar la clonalidad de las células T ?

Como las células T maduras, los genes del TCR están rearreglados en las neoplasias de células T periféricas

La presencia/ausencia de genes TCR rearreglados idénticamente es útil para discriminar entre proliferaciones de células T monoclonal/policlonal

	Pac.B	Pac.O	FITC	PE	PerCP-Cy5.5	PECY7	APC	APCH 7
Step 1 **(screening tube)**	**CD4** CD20	**CD45**	**CD8** Anti-sIgλ	**CD56** Anti-sIgκ	**CD5**	**Anti-TCRγδ** CD20	**SmCD3**	CD38
Step 2 **(T-cell clonality study)**	CD4	-	TCR-Vβ"A" + TCR-Vβ"B"	TCR-Vβ"B" + TCR-Vβ"C"	CD3	-	CD8	-

IOT **TCR-Vbeta** KIT: Reagent composition

"TUBE"	VB Family	Fluorochrome	Clone	Isotype (species)
A	VB 5.3	PE	3D11	IgG1 (mouse)
	VB 7.1	PE + FITC	ZOE	IgG2a (mouse)
	VB 3	FITC	CH92	IgM (mouse)
B	VB 9	PE	FIN9	IgG2a (mouse)
	VB 17	PE + FITC	E17.5F3	IgG1 (mouse)
	VB 16	FITC	TAMAYA1.2	IgG1 (mouse)
C	VB 18	PE	BA62.6	IgG1 (mouse)
	VB 5.1	PE + FITC	IMMU157	IgG2a (mouse)
	VB 20	FITC	ELL1.4	IgG (mouse)
D	VB 13.1	PE	IMMU222	IgG2b (mouse)
	VB 13.6	PE + FITC	JU74.3	IgG1 (mouse)
	VB 8	FITC	56C5.2	IgG2a (mouse)
E	VB 5.2	PE	36213	IgG1 (mouse)
	VB 2	PE + FITC	MPB2D5	IgG1 (mouse)
	VB 12	FITC	VER2.32	IgG2a (mouse)
F	VB 23	PE	AF23	IgG1 (mouse)
	VB 1	PE + FITC	BL37.2	IgG1 (rat)
	VB 21.3	FITC	IG125	IgG2a (mouse)
G	VB 11	PE	C21	IgG2a (mouse)
	VB 22	PE + FITC	IMMU546	IgG1 (mouse)
	VB 14	FITC	CAS1.1.3	IgG1 (mouse)
H	VB 13.2	PE	H132	IgG1 (mouse)
	VB 4	PE + FITC	WJF24	IgM (rat)
	VB 7.2	FITC	ZIZOU4	IgG2a (mouse)

EuroFlow

	Pac.B	Pac.O	FITC	PE	PerCP-Cy5.5	PECY7	APC	APCH 7
Step 1 **(screening tube)**	**CD4** CD20	**CD45**	**CD8** Anti-sIgλ	**CD56** Anti-sIgκ	**CD5**	**Anti-TCRγδ** CD20	**SmCD3**	CD38
Step 2 **(T-cell clonality study)**	CD4	-	TCR-Vβ"A" + TCR-Vβ"B"	TCR-Vβ"B" + TCR-Vβ"C"	CD3	-	CD8	-

IOT **TCR-Vbeta** KIT: Reagent composition

"TUBE"	VB Family	Fluorochrome	Clone	Isotype (species)
A	VB 5.3	PE	3D11	IgG1 (mouse)
	VB 7.1	PE + FITC	ZOE	IgG2a (mouse)
	VB 3	FITC	CH92	IgM (mouse)
B	VB 9	PE	FIN9	IgG2a (mouse)
	VB 17	PE + FITC	E17.5F3	IgG1 (mouse)
	VB 16	FITC	TAMAYA1.2	IgG1 (mouse)
C	VB 18	PE	BA62.6	IgG1 (mouse)
	VB 5.1	PE + FITC	IMMU157	IgG2a (mouse)
	VB 20	FITC	ELL1.4	IgG (mouse)
D	VB 13.1	PE	IMMU222	IgG2b (mouse)
	VB 13.6	PE + FITC	JU74.3	IgG1 (mouse)
	VB 8	FITC	56C5.2	IgG2a (mouse)
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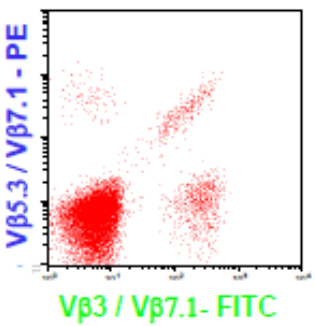
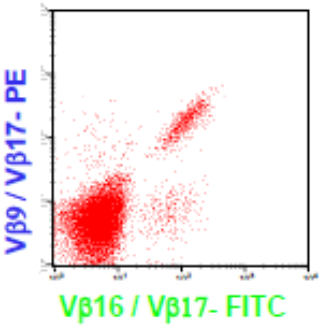
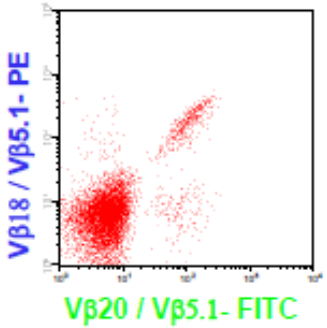
	VB 2	PE + FITC	MPB2D5	IgG1 (mouse)
	VB 12	FITC	VER2.32	IgG2a (mouse)
F	VB 23	PE	AF23	IgG1 (mouse)
	VB 1	PE + FITC	BL37.2	IgG1 (rat)
	VB 21.3	FITC	IG125	IgG2a (mouse)
G	VB 11	PE	C21	IgG2a (mouse)
	VB 22	PE + FITC	IMMU546	IgG1 (mouse)
	VB 14	FITC	CAS1.1.3	IgG1 (mouse)
H	VB 13.2	PE	H132	IgG1 (mouse)
	VB 4	PE + FITC	WJF24	IgM (rat)
	VB 7.2	FITC	ZIZOU4	IgG2a (mouse)

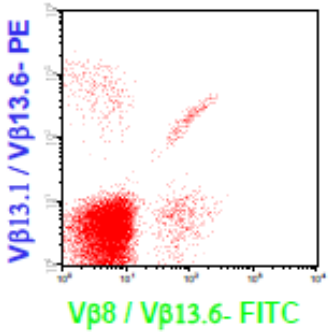
EuroFlow

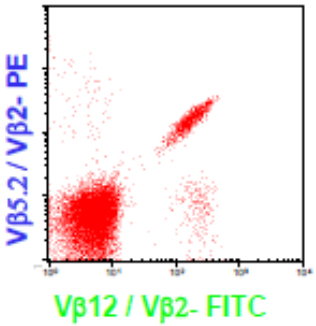
NORMAL PB CD4+ T-CELLS: TCRVβ REPERTOIRE

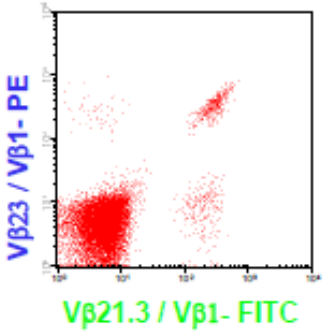


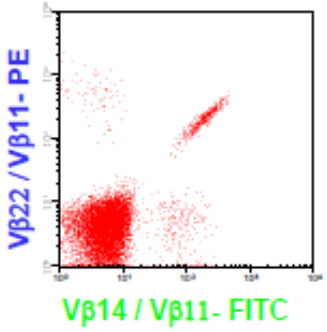


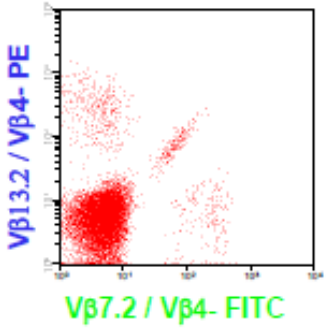






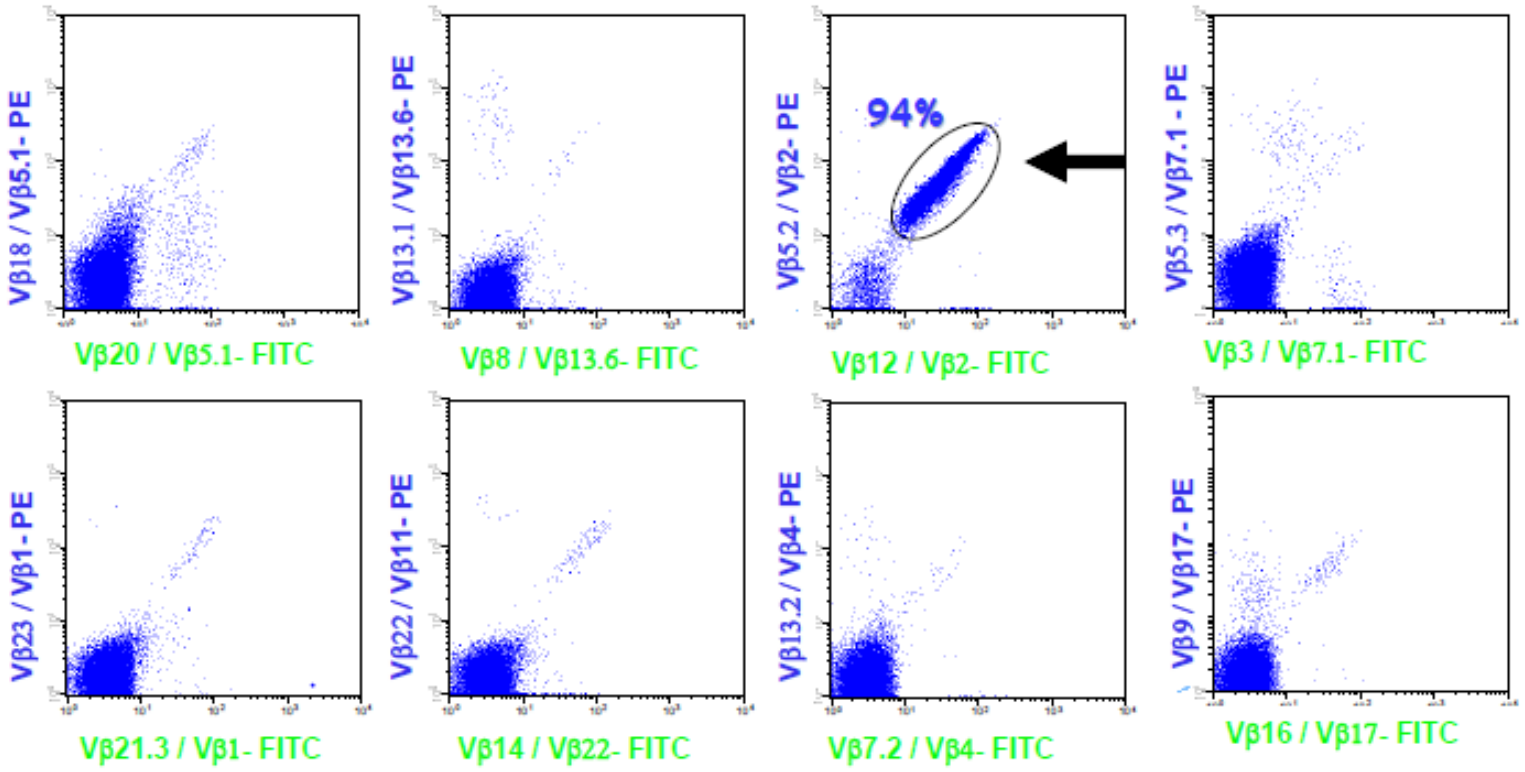




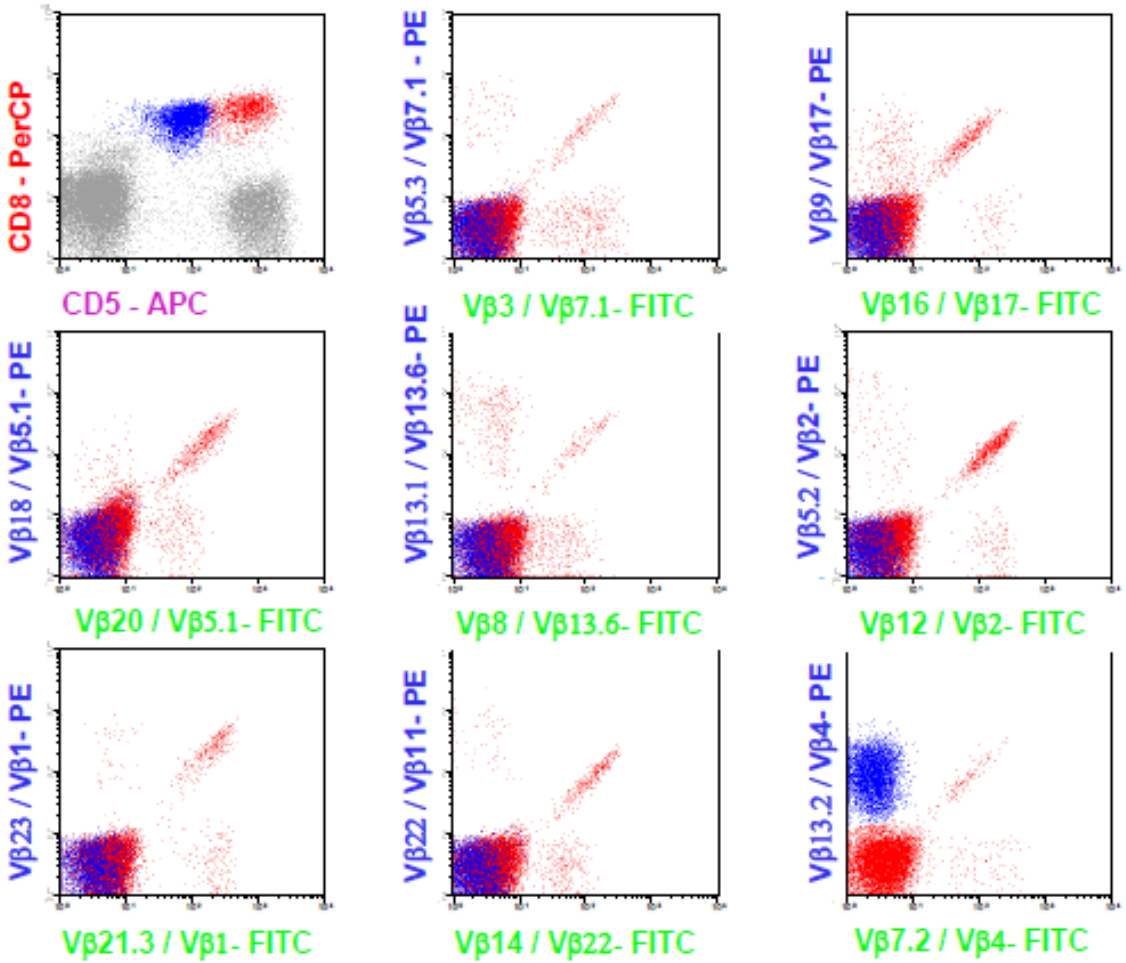


T-CELL LEUKEMIAS: PHENOTYPIC ANALYSIS OF THE TCRVβ REPERTOIRE

CD4+ TCRαβ+ gated CELLS



T-CELL LEUKEMIAS: PHENOTYPIC ASSESSMENT OF CLONALITY



Técnicas para el análisis del repertorio del TCRaß

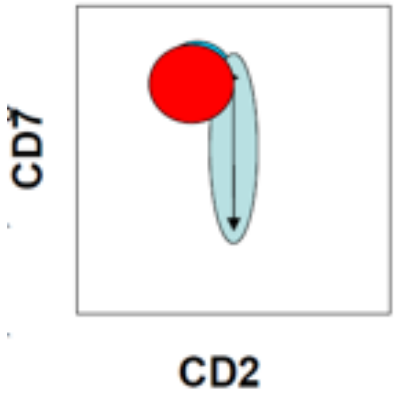
	Anticuerpos V β	RT-PCR
Ventajas	Rápida Cuantifica Multiparamétrica	>98% de todos los Vβ Análisis de la región J
Desventajas	Detecta 65-70% de los dominios Vβ No aporta información de la región J Cara	Laboriosa Relativamente lenta Cara No cuantifica Pseudo-oligo-monoclonalidad

Seguimiento de SLP-T

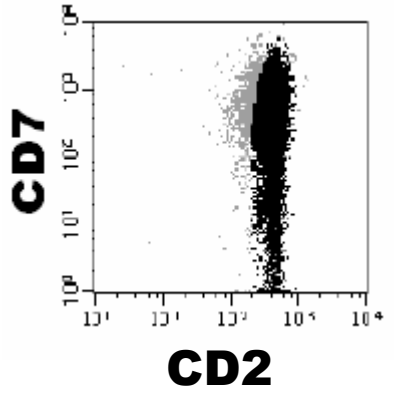
TCLPD-	PacB	PacO	FITC	PE	PerCP Cy5.5	PECY7	APC	APC H7	AIM
1	CD4	CD45	CD7	CD26	smCD3	CD2	CD28	CD8	Identificación de síndrome Sézary
2	CD4	CD45	CD27	CCR7	smCD3	CD45RO	CD45RA	CD8	Evaluación estado de maduración
3	CD4	CD45	CD5	CD25	smCD3	HLADR	cyTCL1	CD8	identificación de T-PLL
4	CD4	CD45	CD57	CD30	smCD3		CD11c	CD8	fenotipo citotóxico & ALCL
5	CD4	CD45	cyPerf	cyGrz	smCD3	CD16	CD94	CD8	fenotipo citotóxico & T-LGL
6	CD4	CD45		CD279	smCD3			CD8	identificación de AITL

Backbone markers

TCLPD-	PacB	PacO	FITC	PE	PerCP Cy5.5	PECY7	APC	APC H7	AIM
1	CD4	CD45	CD7	CD26	smCD3	CD2	CD28	CD8	Identificación de síndrome Sézary

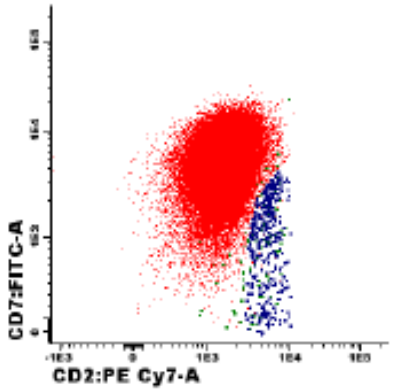


Patrón normal de células T

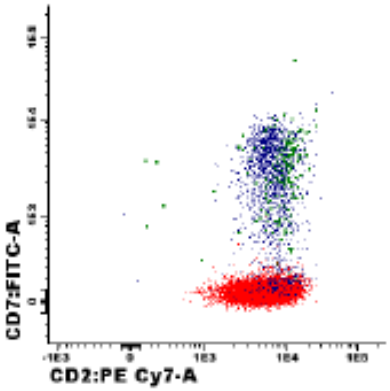


Patrón alterado

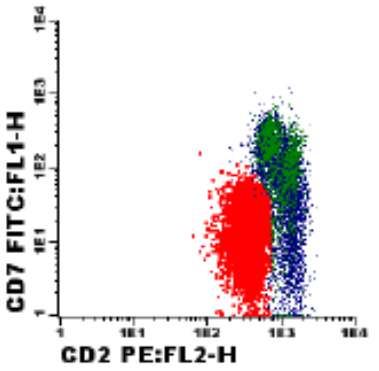
T-PLL (CD7++CD2+(débil)



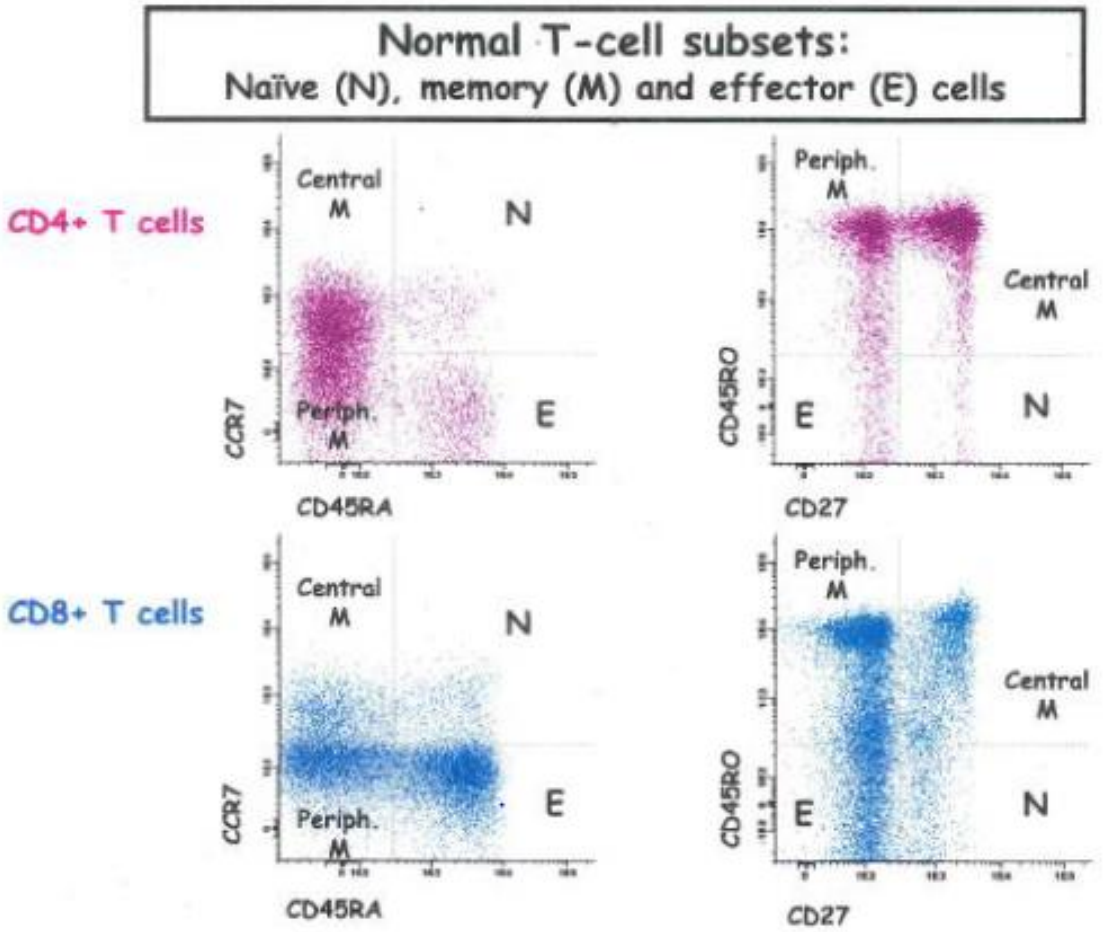
SS (CD7- (o débil) CD2+ (o débil))



LGL (CD7+(débil)CD2+(débil))

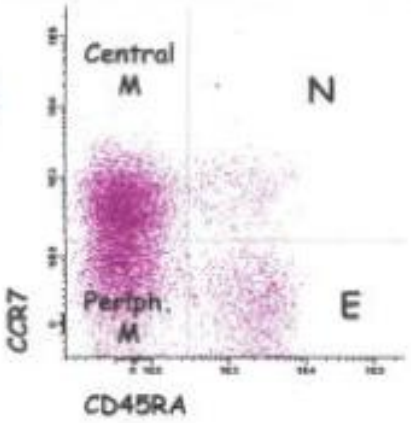


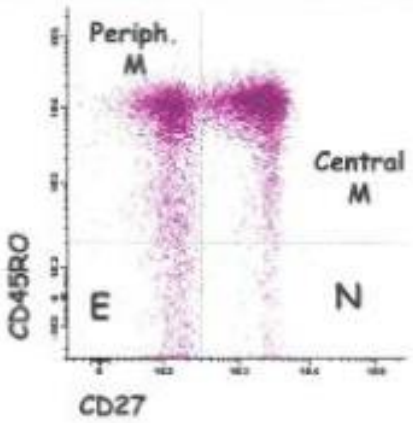
TCLPD-	PacB	PacO	FITC	PE	PerCP Cy5.5	PECY7	APC	APC H7	AIM
1	CD4	CD45	CD7	CD26	smCD3	CD2	CD28	CD8	Identificación de síndrome Sézary
2	**CD4**	**CD45**	CD27	CCR7	**smCD3**	CD45RO	CD45RA	**CD8**	**Evaluación estado de maduración**

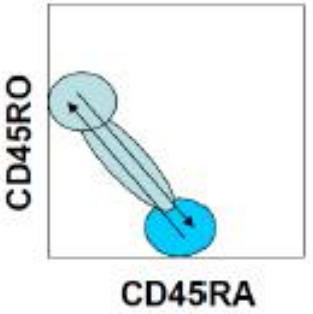


Normal T-cell subsets:
Naïve (N), memory (M) and effector (E) cells

CD4+ T cells

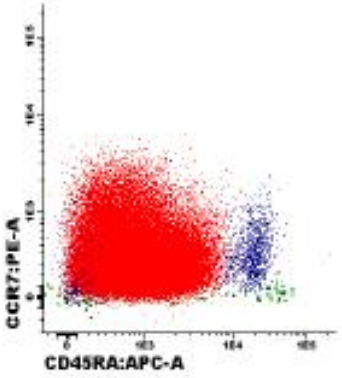


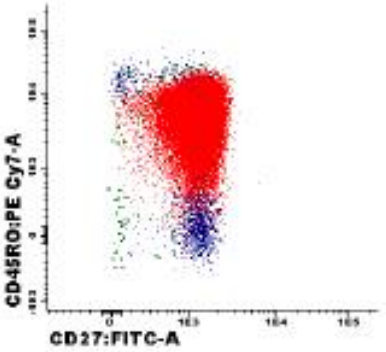


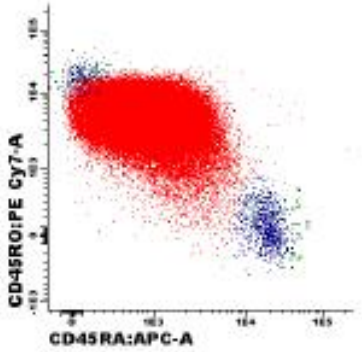


CD4+ T-PLL

-> central memory

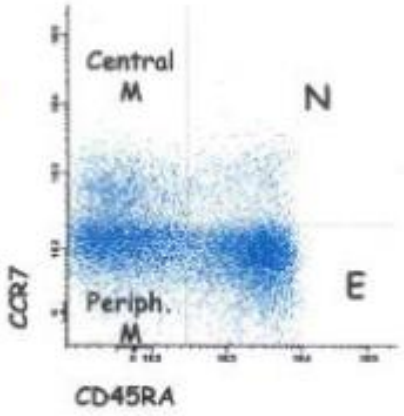


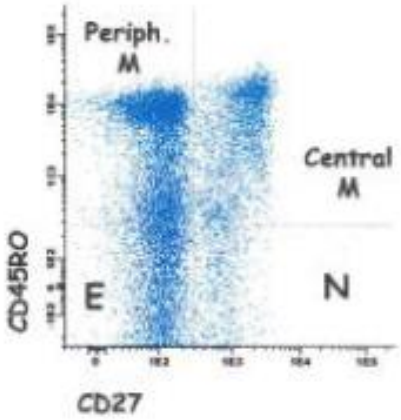


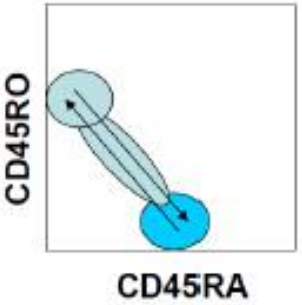


Normal T-cell subsets:
Naïve (N), memory (M) and effector (E) cells

CD8+ T cells

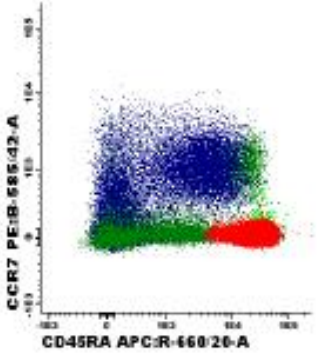


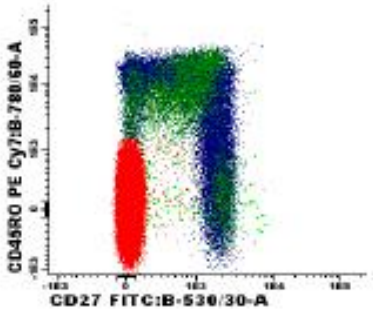


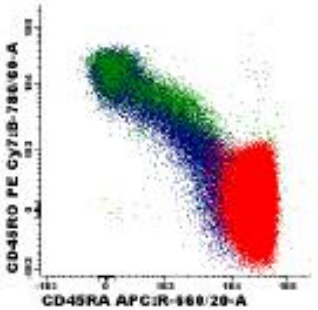


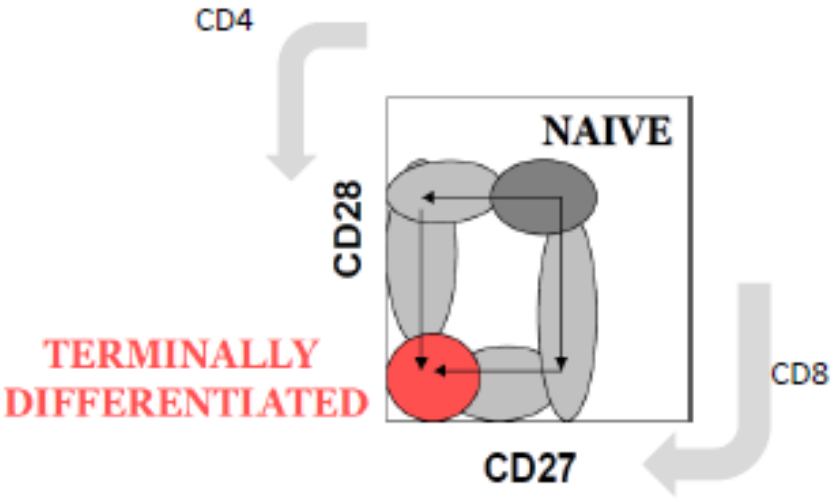
CD8+LGL

-> effector T cell



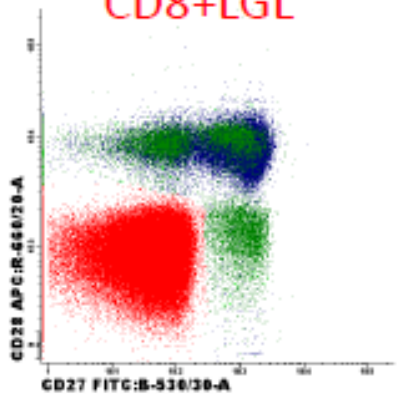






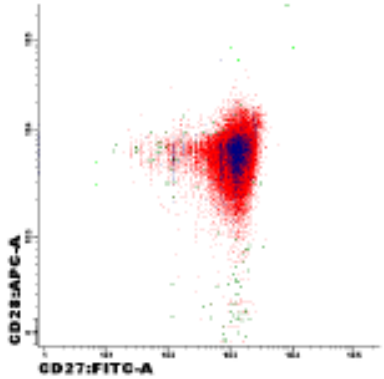
Expresión de Ags co-estimuladores CD27 y CD28

CD8+LGL



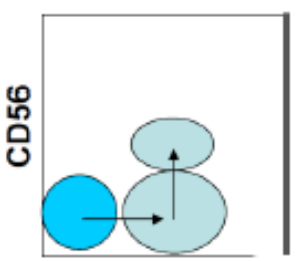
terminally differentiated effector T cell

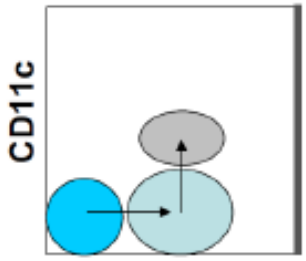
CD4+ T-PLL

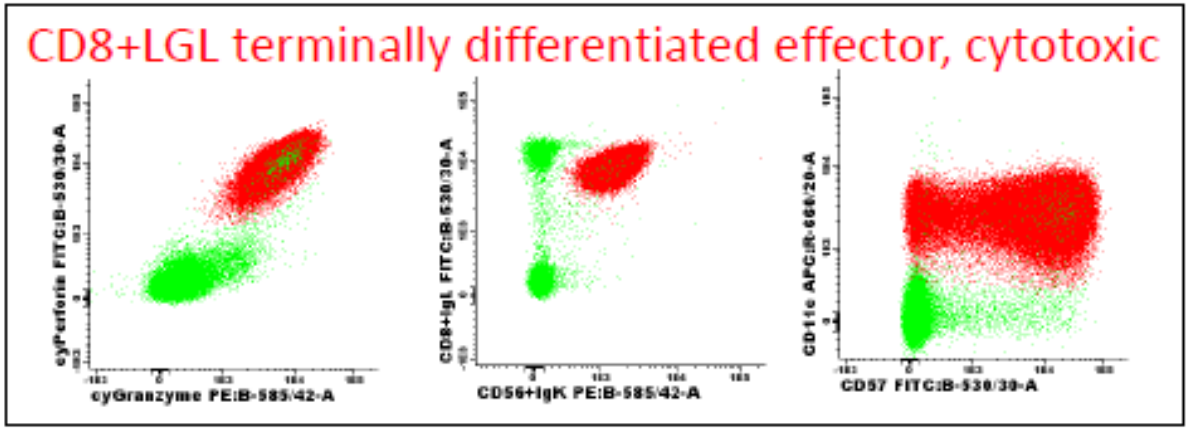


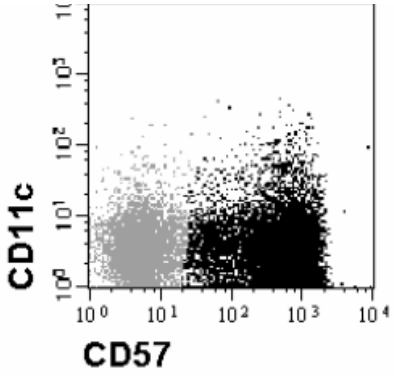
central memory

TCLPD-	PacB	PacO	FITC	PE	PerCP Cy5.5	PECY7	APC	APC H7	AIM
1	CD4	CD45	CD7	CD26	smCD3	CD2	CD28	CD8	Identificación de síndrome Sézary
2	CD4	CD45	CD27	CCR7	smCD3	CD45RO	CD45RA	CD8	Evaluación estado de maduración
3	CD4	CD45	CD5	CD25	smCD3	HLADR	cyTCL1	CD8	identificación de T-PLL
4	**CD4**	**CD45**	CD57	CD30	**smCD3**		CD11c	**CD8**	**fenotipo citotóxico & ALCL**
5	**CD4**	**CD45**	cyPerf	cyGrz	**smCD3**	CD16	CD94	**CD8**	**fenotipo citotóxico & T-LGL**

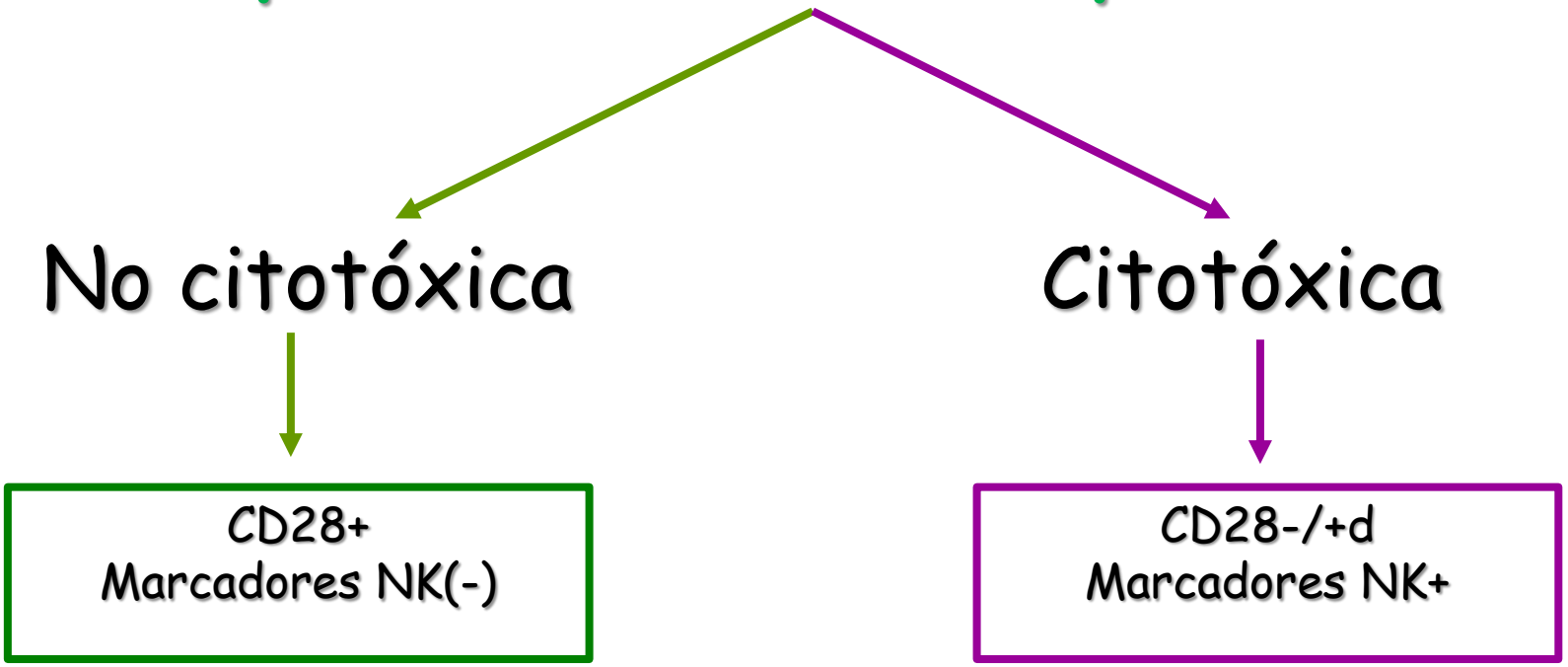








Neoplasias de células T periféricas



Neoplasias de células T y NK maduras WHO 2008

Mature T-cell Leukemias (leukemic/disseminated)

- T-cell prolymphocytic leukemia (T-PLL)
- T-cell LGL leukemia (T-LGL)
- Chronic LPD of NK cells*
- Aggressive NK-cell leukemia
- EBV+ T-cell LPD of childhood
- Adult T-cell lymphoma/leukemia (HTLV1+) (ATLL)

Extranodal T-cell Lymphomas

- Extranodal NK/T-cell lymphoma, nasal type
- Enteropathy-associated T-cell lymphoma (EATL)
- Hepatosplenic T-cell lymphoma (HSTL)
- Subcutaneous panniculitis-like T-cell lymphoma (SPTCL)

Nodal T-cell Lymphomas

- Angioimmunoblastic T-cell lymphoma (AITL)
- Anaplastic large cell lymphoma, *ALK*+ (ALCL)
- Anaplastic large cell lymphoma, *ALK*- * (ALCL)
- Peripheral T-cell lymphoma, NOS (PTCL-NOS)

Cutaneous T-cell Lymphomas (extranodal)

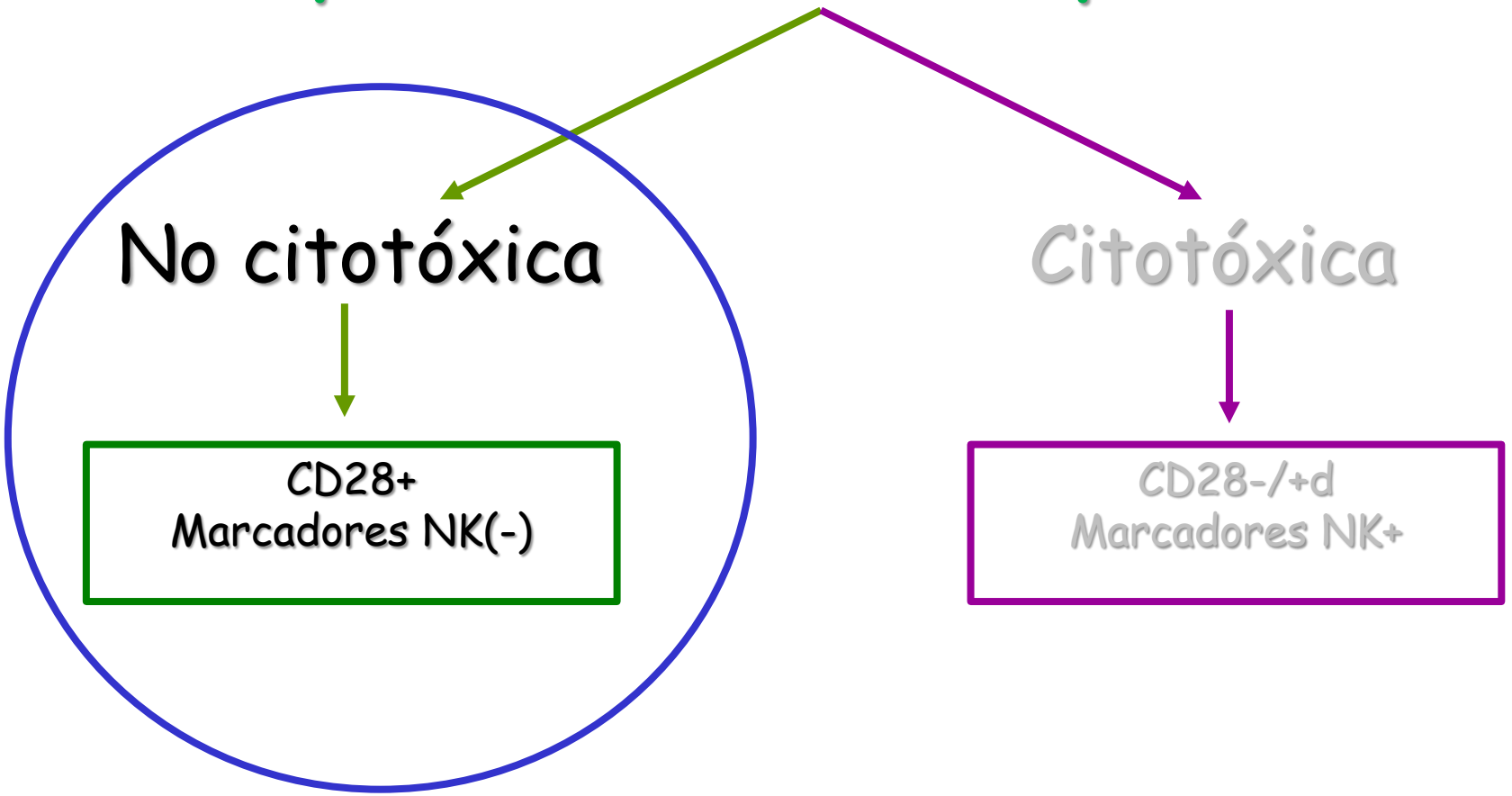
- Sézary syndrome (SS)/ Mycosis fungoides (MF)
- Primary cutaneous CD30+ TCLPD
- Primary cutaneous PTCLs, rare subtypes

fenotipo

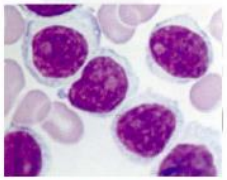
- citotoxico
- no citotoxico
- En su mayoria citotoxico
- variable

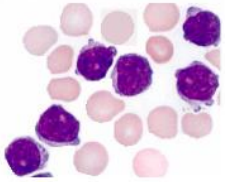
* entidad provisional

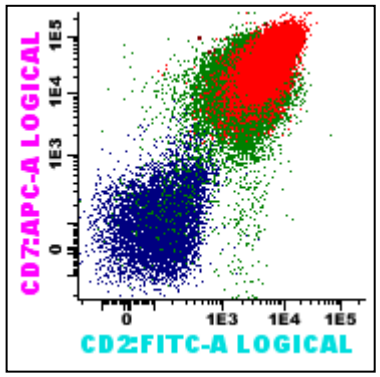
Neoplasias de células T periféricas

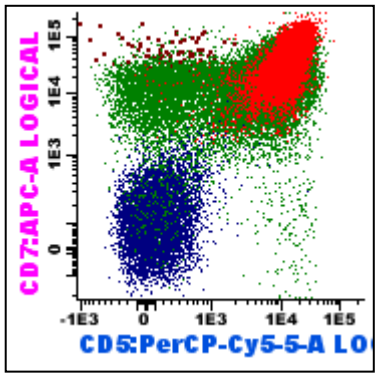


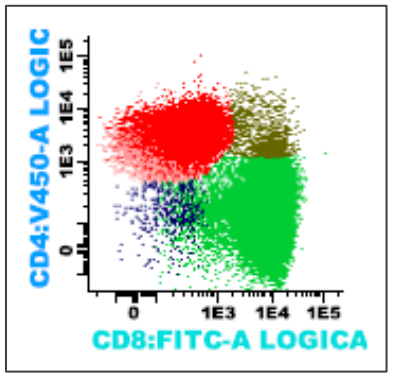
Leucemia prolinfocítica T

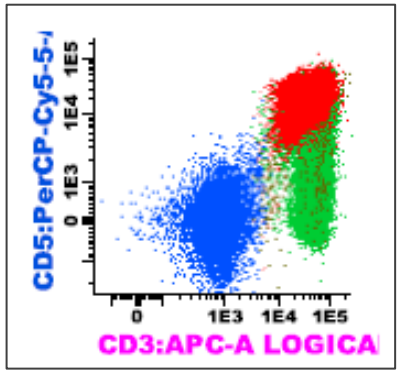


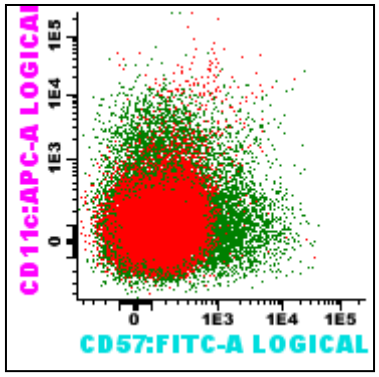


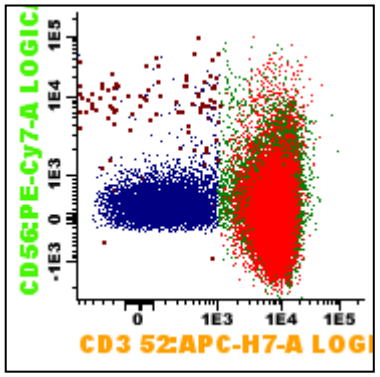


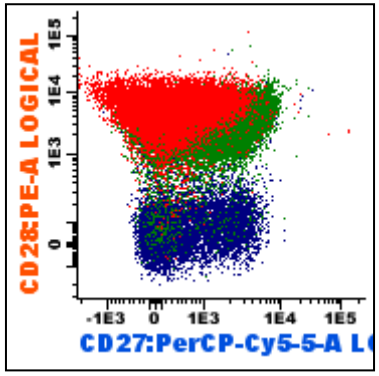


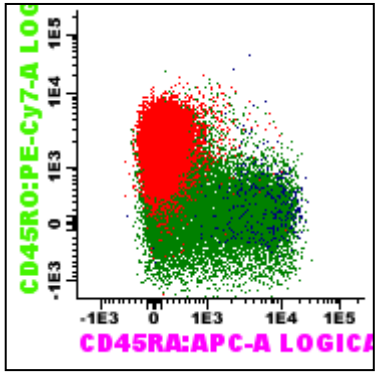






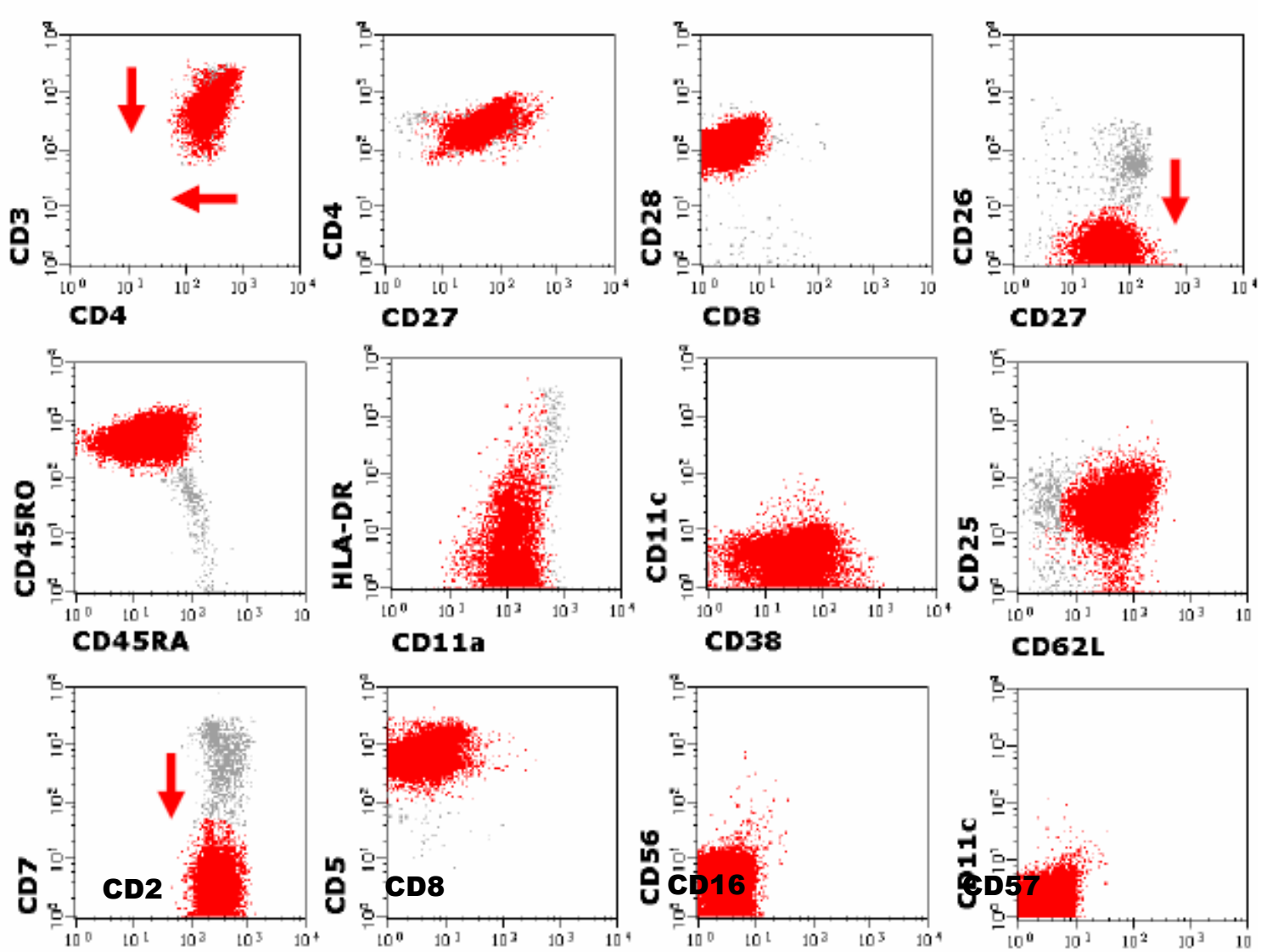




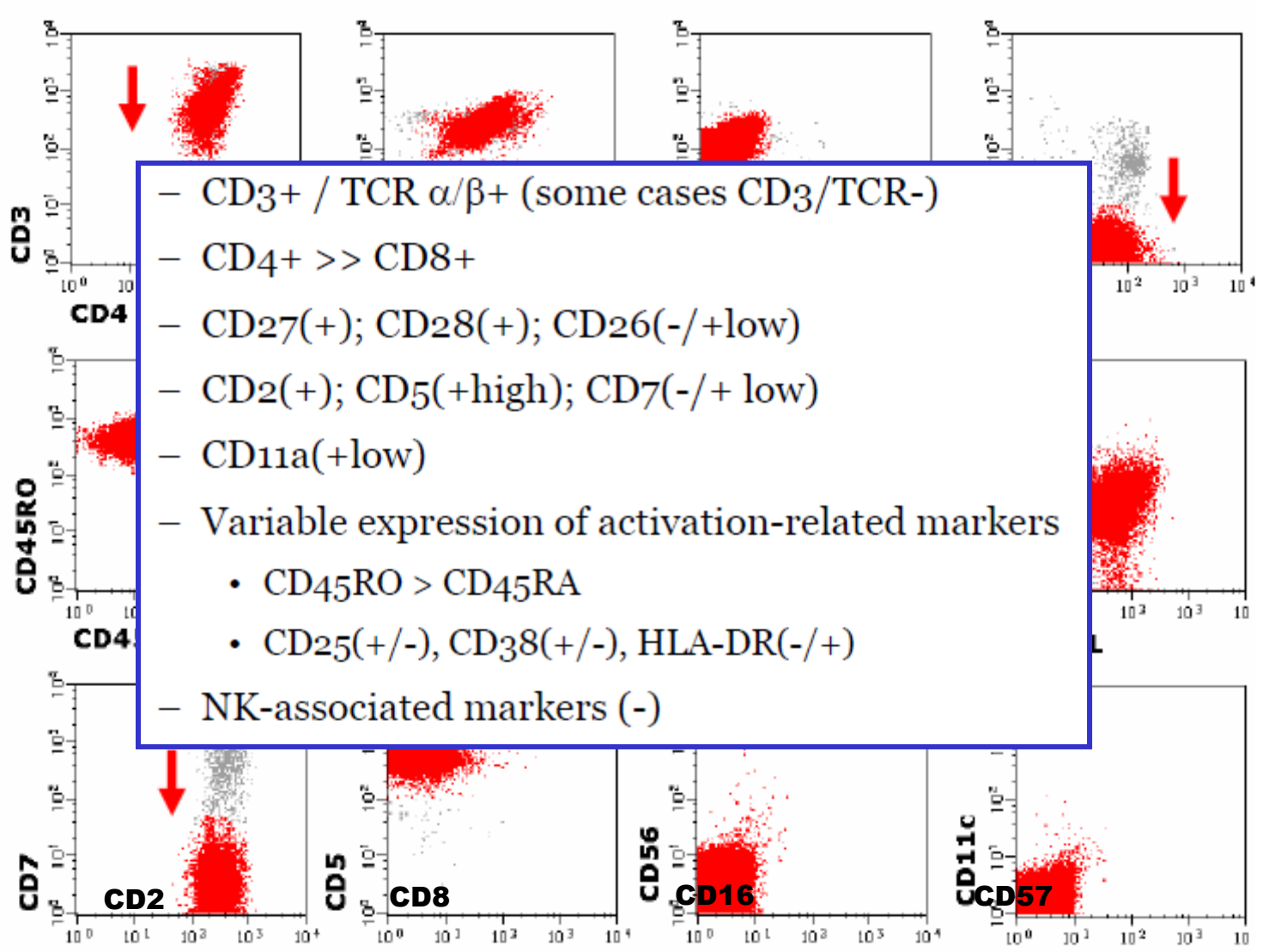


- CD3+ TCR α/β+
- CD4+ >> CD8+
- CD27(+high); CD28(+high); CD26(+)
- CD2(+low); CD5(+high); CD7(+high)
- CD11a(+low)
- Variable expression of activation-related markers
  - CD45RA / CD45RO (+ / - 5%) (+ / + 45%) (- / + 50%) (RO > RA)
  - CD25(+/-), CD38(+/-), HLA-DR(-/+; <10% cases)
- NK-associated markers (-) - CyTCL1+

Linfoma T periférico no citotóxico

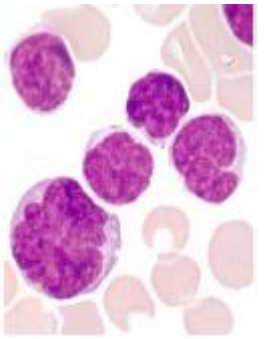


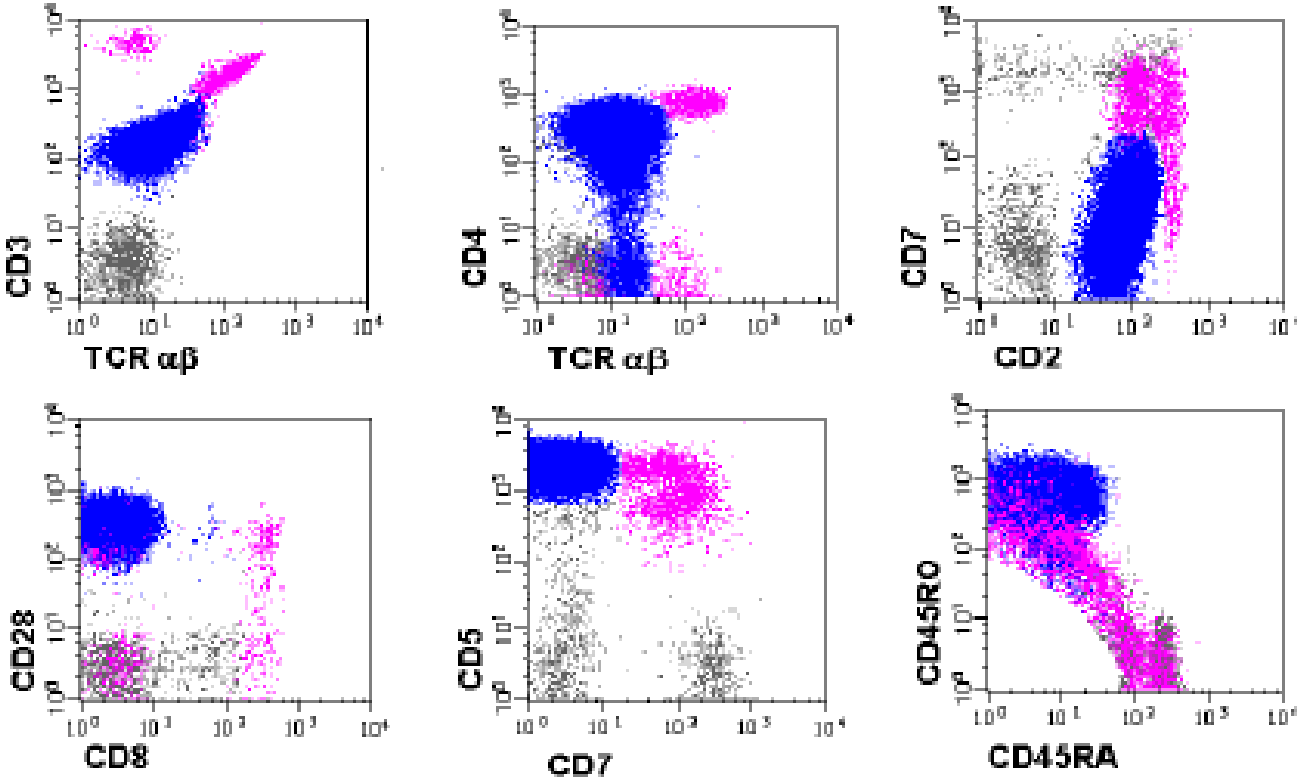
Linfoma T periférico no citotóxico



- CD3+ / TCR α/β+ (some cases CD3/TCR-)
- CD4+ >> CD8+
- CD27(+); CD28(+); CD26(-/+low)
- CD2(+); CD5(+high); CD7(-/+ low)
- CD11a(+low)
- Variable expression of activation-related markers
  - CD45RO > CD45RA
  - CD25(+/-), CD38(+/-), HLA-DR(-/+)
- NK-associated markers (-)

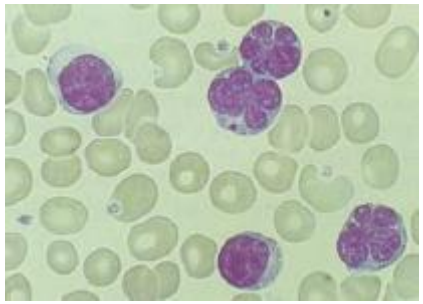
Síndrome de Sézary/Micosis fungoide

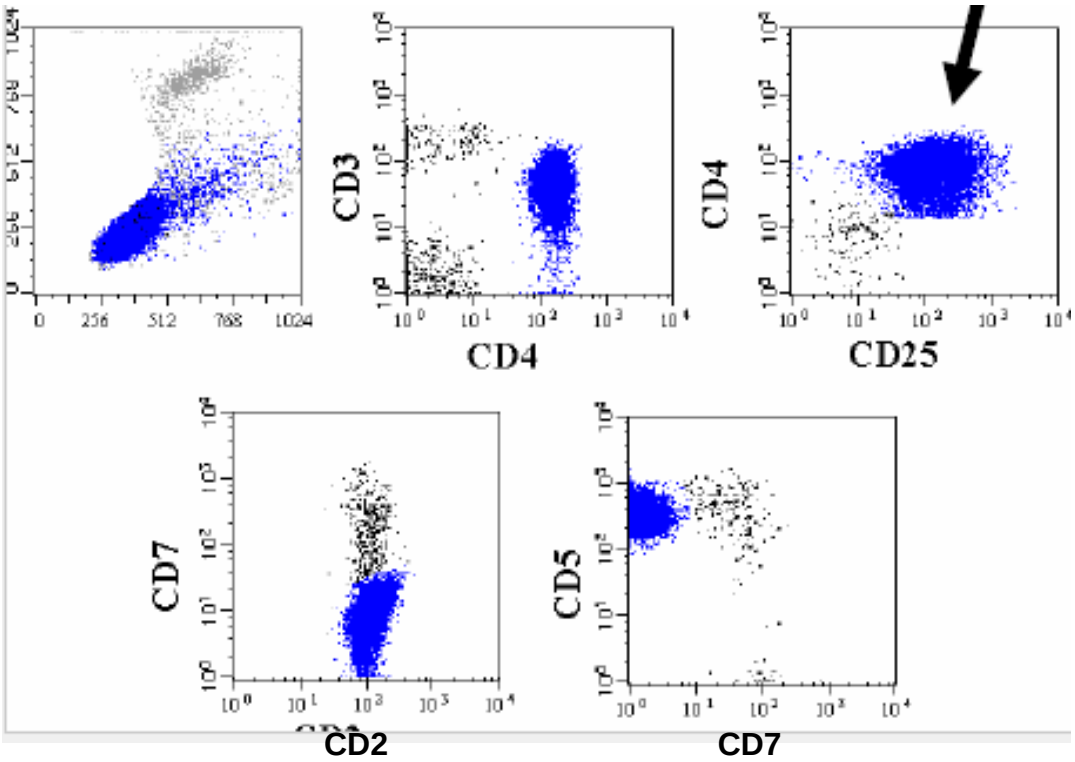




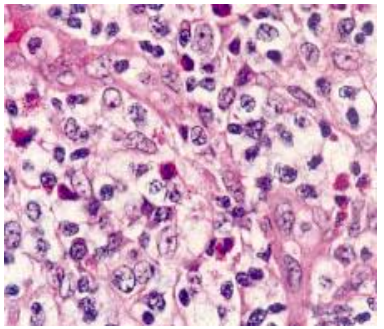
CD3 $^{-/+d}$ **TCRαβ**$^{-/+d}$ **CD4**$^{-/+d}$ CD2$^{-/+d}$ CD5^{+high}
CD7$^{-/+d}$ CD45RO^{+} CD45RA$^{-/+d}$ CD26$^{-/+d}$ CD27^{+}
CD28^{+} CD25$^{-/+d}$ HLA-DR$^{-/+d}$ CD38 $^{-/+d}$ NKa^{-} CLA^{+}

Leucemia/linfoma T del adulto HTLV1+



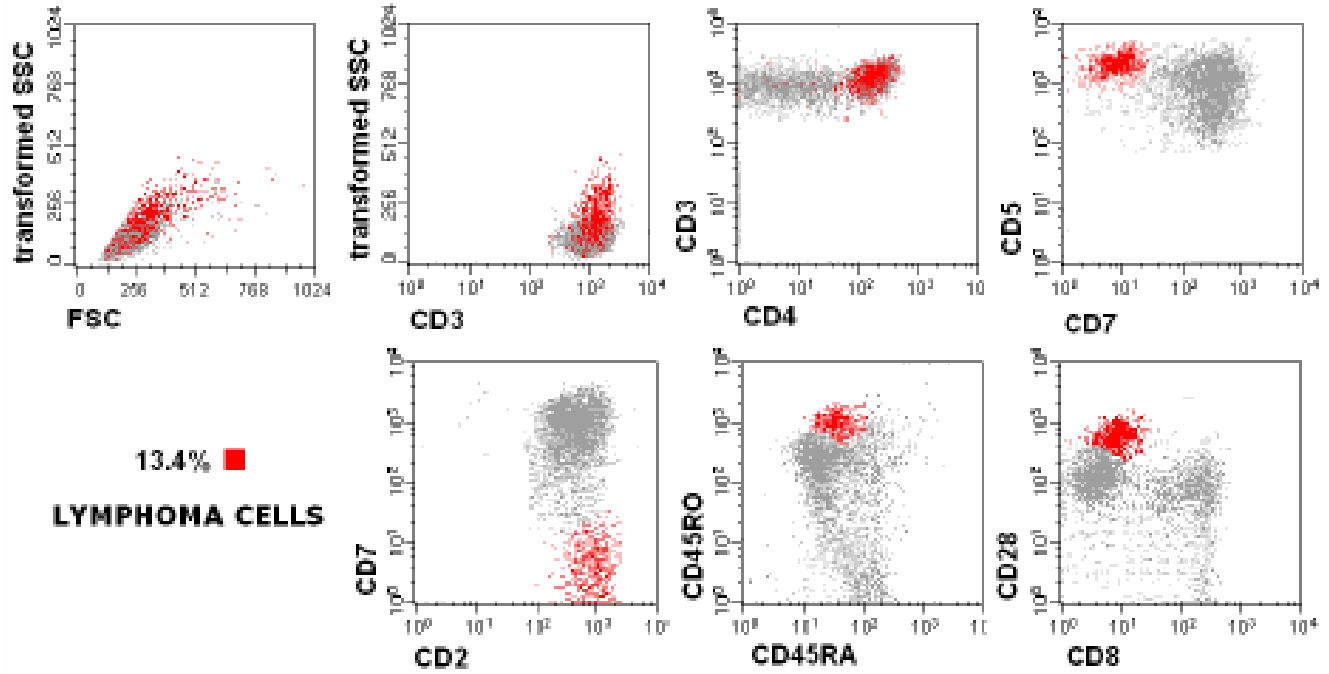


Linfoma angioinmunoblástico



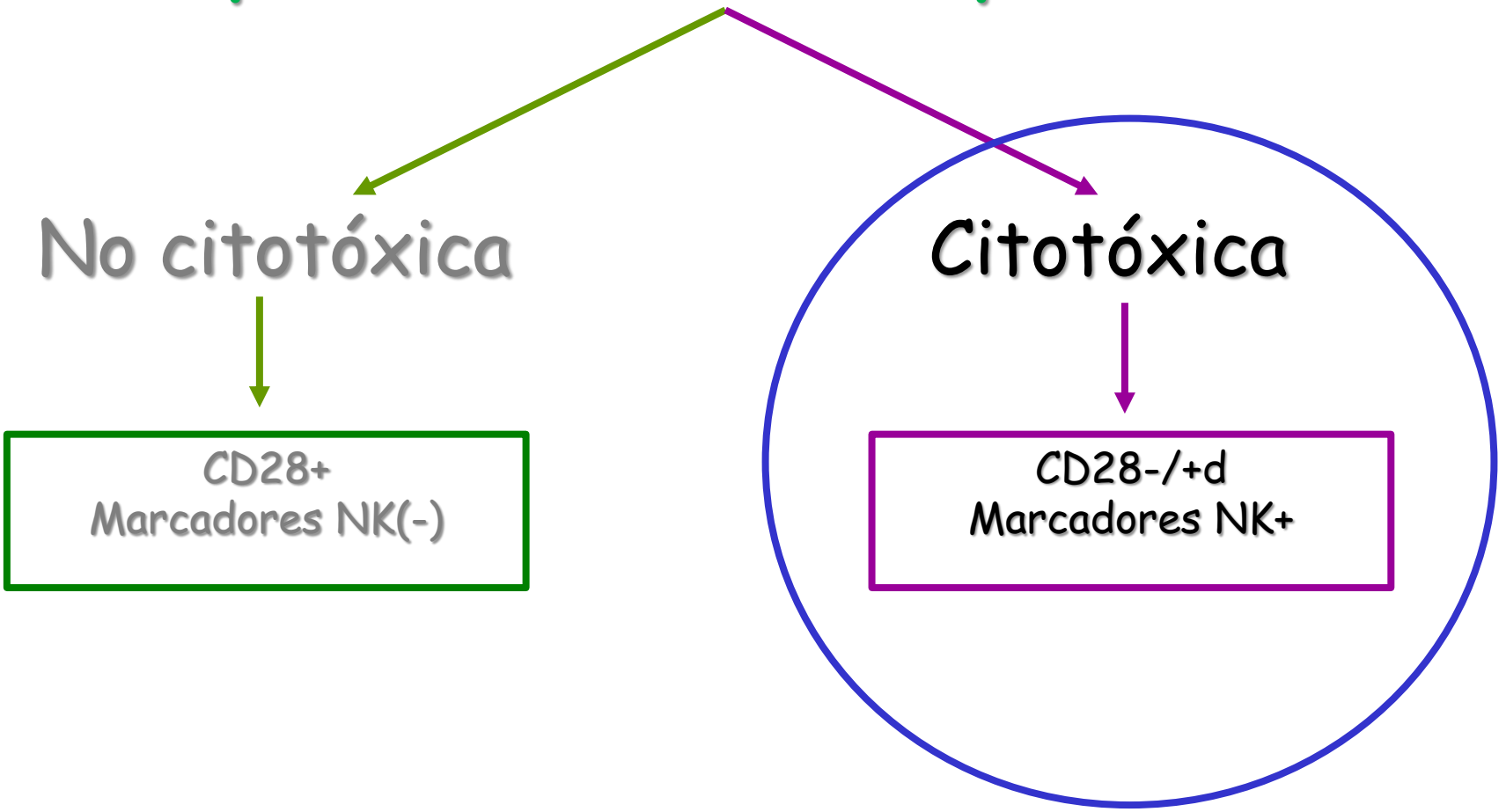
CD3, CD4 (sometimes CD10+), germinal center-T cells.

ABNORMAL CD4+ T CELLS

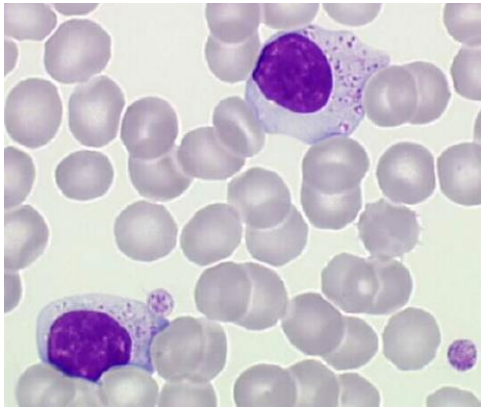


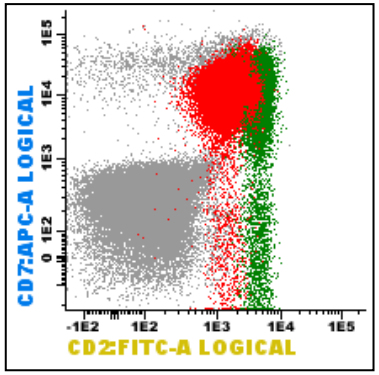
Se asocia frecuentemente a hiperplasia linfoide B y a infección por EBV.

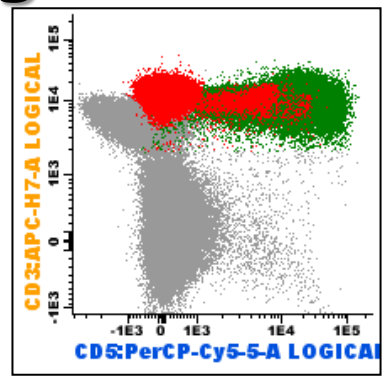
Neoplasias de células T periféricas

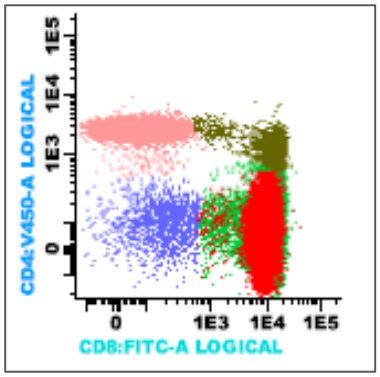


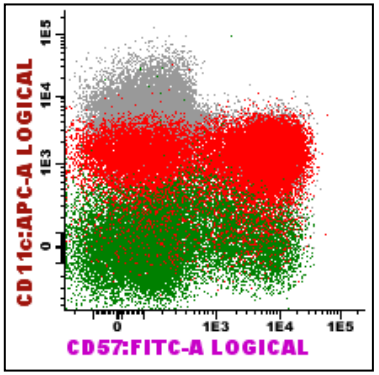
Leucemia de linfocitos T grandes granulares LGL

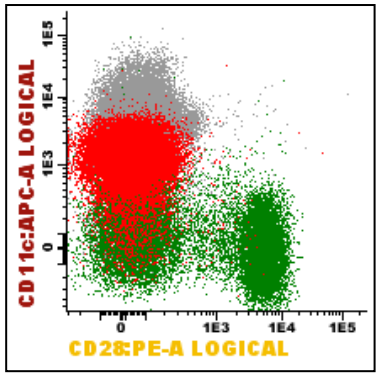


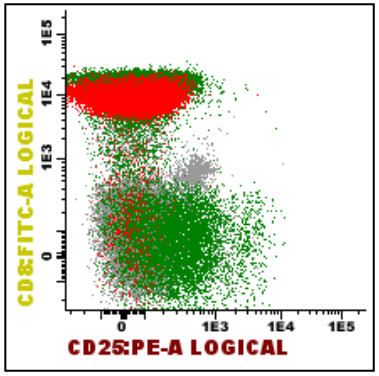


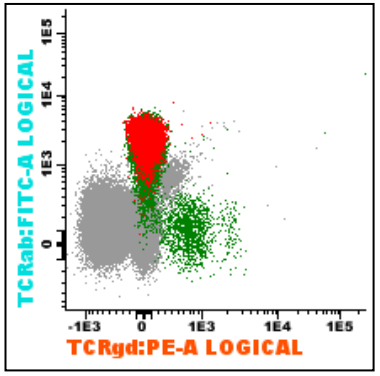


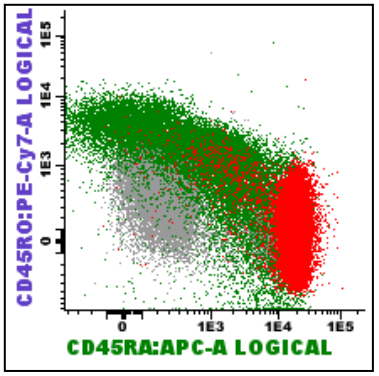


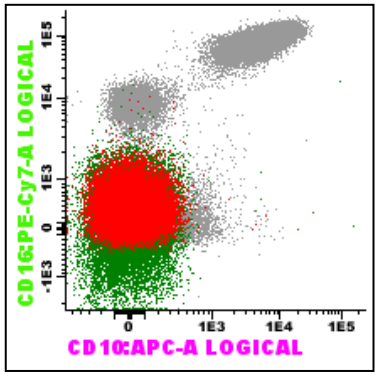


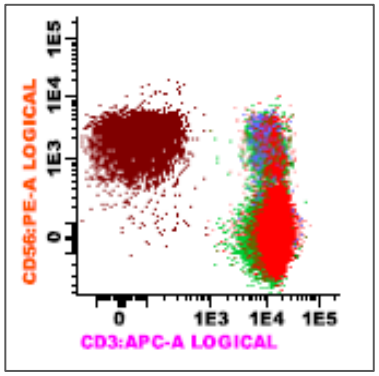




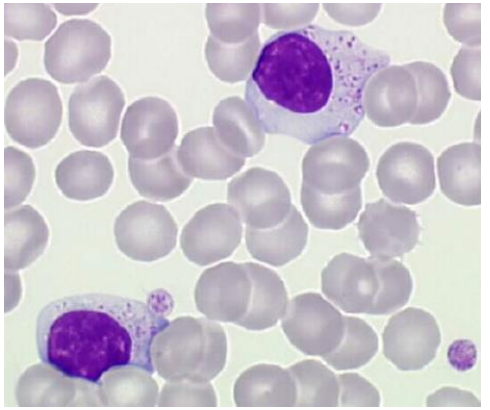


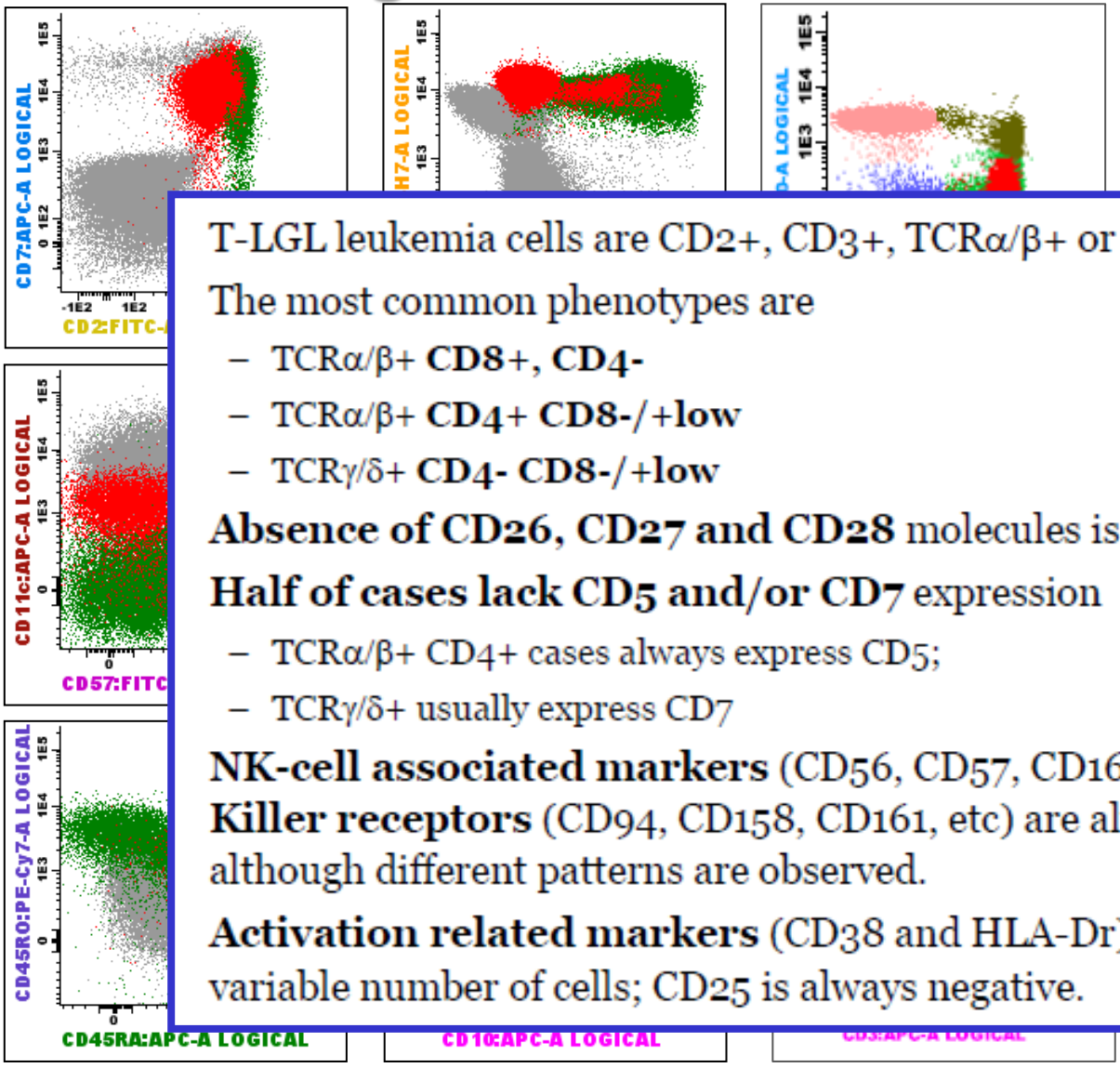






Leucemia de linfocitos T grandes granulares LGL





T-LGL leukemia cells are CD2+, CD3+, TCRα/β+ or TCRγ/δ+

The most common phenotypes are

- TCRα/β+ **CD8+, CD4-**
- TCRα/β+ **CD4+ CD8-/+low**
- TCRγ/δ+ **CD4- CD8-/+low**

Absence of CD26, CD27 and CD28 molecules is the role

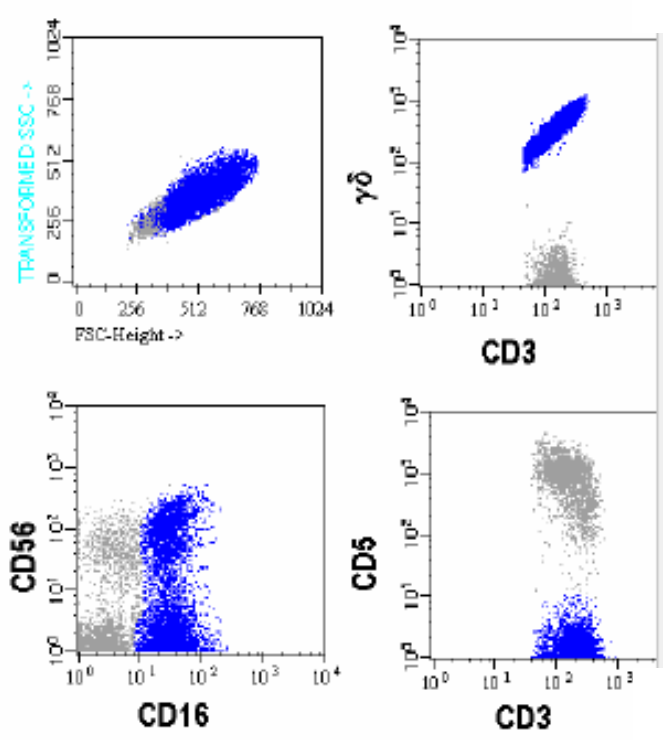
Half of cases lack CD5 and/or CD7 expression

- TCRα/β+ CD4+ cases always express CD5;
- TCRγ/δ+ usually express CD7

NK-cell associated markers (CD56, CD57, CD16, CD11b, CD11c) and **Killer receptors** (CD94, CD158, CD161, etc) are always expressed, although different patterns are observed.

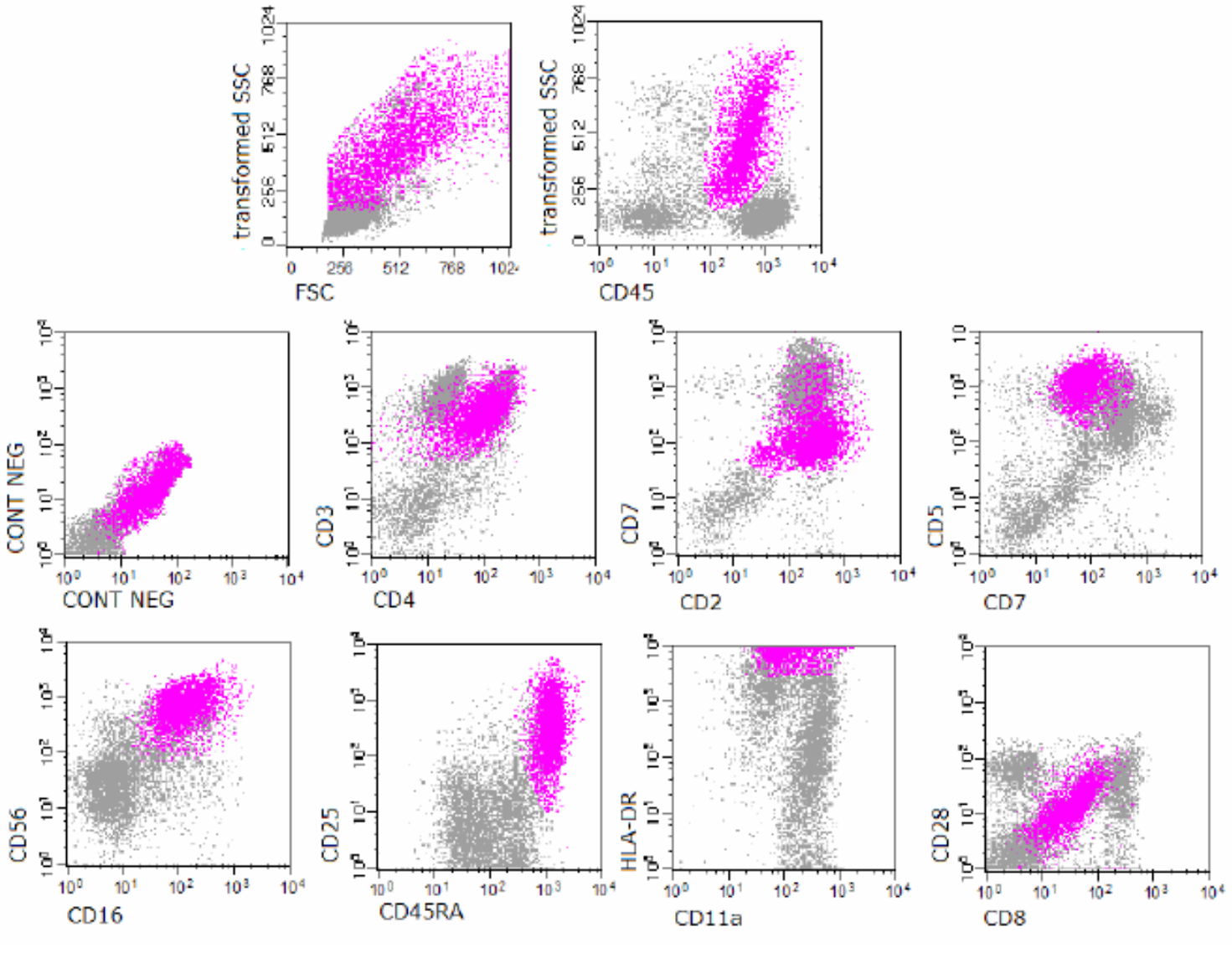
Activation related markers (CD38 and HLA-Dr) are expressed in a variable number of cells; CD25 is always negative.

Linfoma T hepatoesplénico



- The neoplastic cells are usually CD2+, CD7+, CD3+, TCR γ/δ+
- TCR α/β+ variants have been described
- **CD5 is often weak or negative**
- **CD4 and CD8 are usually absent** (weak CD8 expression may be observed)
- **CD16 and CD56** are usually positive (CD57 is frequently negative)
- **TIA-1** (cytotoxic granule-associated proteins) is present in virtually all cases.
- Approximately **50% lack granzyme B and or perforin**, indicating a non-activated cytolytic T-cell phenotype

Linfoma T anáplasico CD30+



Linfoma anaplásico de células grandes asociado a implantes mamarios (BIA-ALCL) OMS 2016

El linfoma a grandes células anaplásico asociado a implantes mamarios (BIA-ALCL) es un tipo infrecuente de linfoma T periférico, CD30 positivo y ALK negativo, que se presenta en la mayor parte de los casos como una colección líquida alrededor de un implante mamario o de su cápsula.

- ✓ Incidencia es variable según la edad: 1/35.000 implantes en mayores de 50 años
  1/12.000 implantes en mayores de 70 años
  1/7.000 implantes en mayores de 75 años
- ✓ Respuesta inmune intensa a la silicona por parte de los linfocitos T, responsables de la inmunidad celular.
- ✓ El BIA-ALCL se presenta habitualmente como una colección líquida (seroma periprotésico) o como una masa capsular y otras manifestaciones como agrandamiento mamario, retracción de la cápsula, ulceración, eritema en piel, adenopatías axilares, sudoración nocturna o fatiga.

Linfoma anaplásico de células grandes asociado a implantes mamarios (BIA-ALCL) OMS 2016

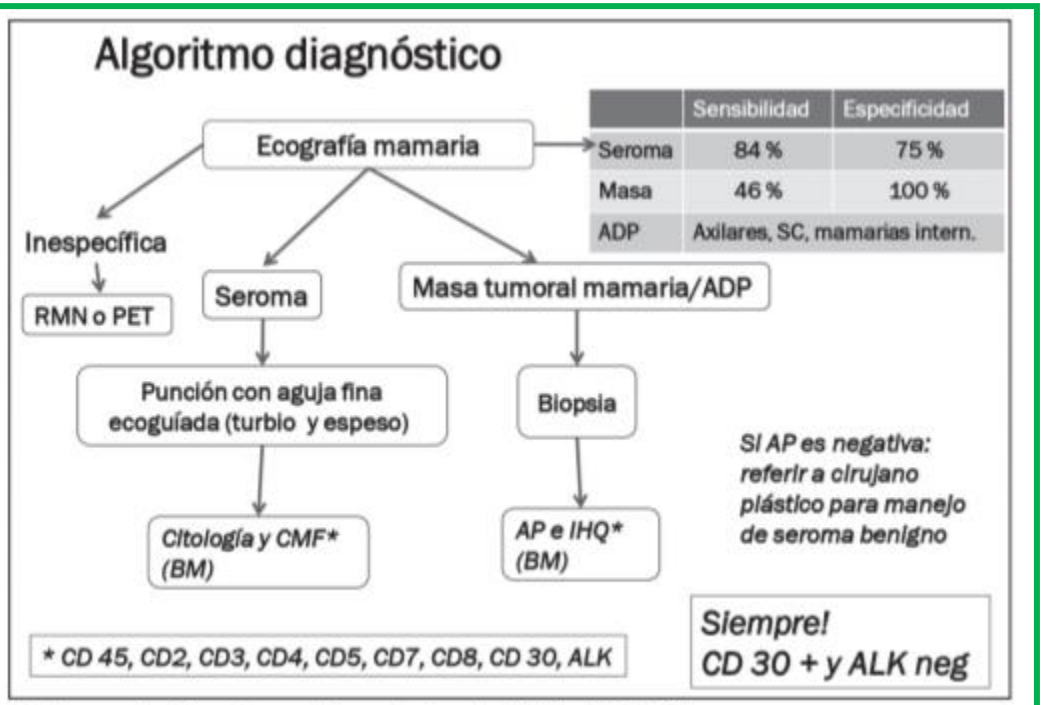

Algoritmo diagnóstico
Ecografía mamaria
Inespecífica
RMN o PET
Seroma
Masa tumoral mamaria/ADP
Punción con aguja fina ecoguíada (turbio y espeso)
Biopsia
Citología y CMF* (BM)
AP e IHQ* (BM)
SI AP es negativa: referir a cirujano plástico para manejo de seroma benigno
* CD 45, CD2, CD3, CD4, CD5, CD7, CD8, CD 30, ALK
Siempre! CD 30 + y ALK neg
Sensibilidad
Especificidad
Seroma 84 % 75 %
Masa 46 % 100 %
ADP Axilares, SC, mamarias intern.

Figura 2. Algoritmo diagnóstico del BIA-ALCL[23].

DIAGNÓSTICO

Pruebas de imágenes:

- **Ecografía**
- **RMN**
- **PET-CT**

PAAF:

- **Citología/IHQ: CD30+, ALK(-)**
- **Citometría de flujo: CD30+**

(marcadores T)

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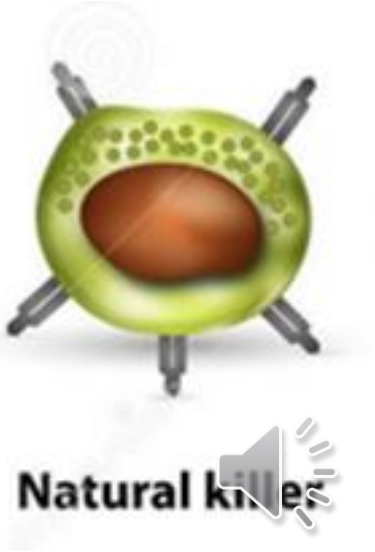
CÉLULAS NK

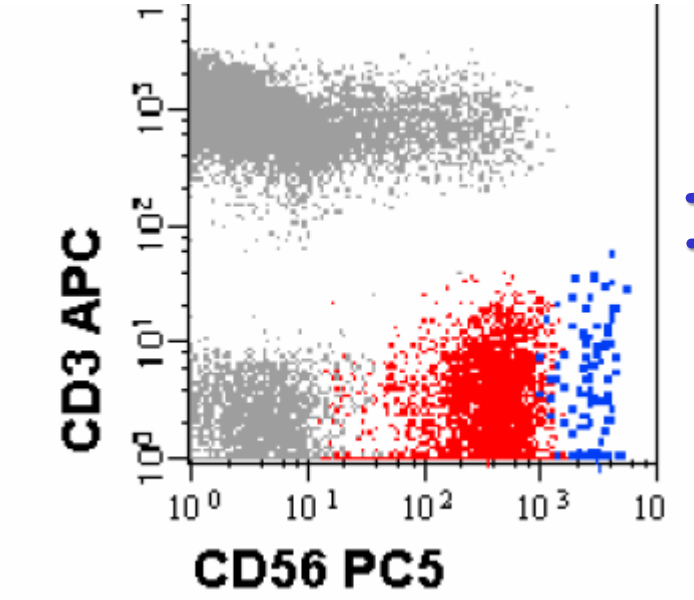


Neoplasias de células NK

- Leucemias de células NK
  - Agresiva
  - Indolente
- Linfoma de célula T/NK extranodal, tipo nasal

Células NK en SP

Citotóxicas

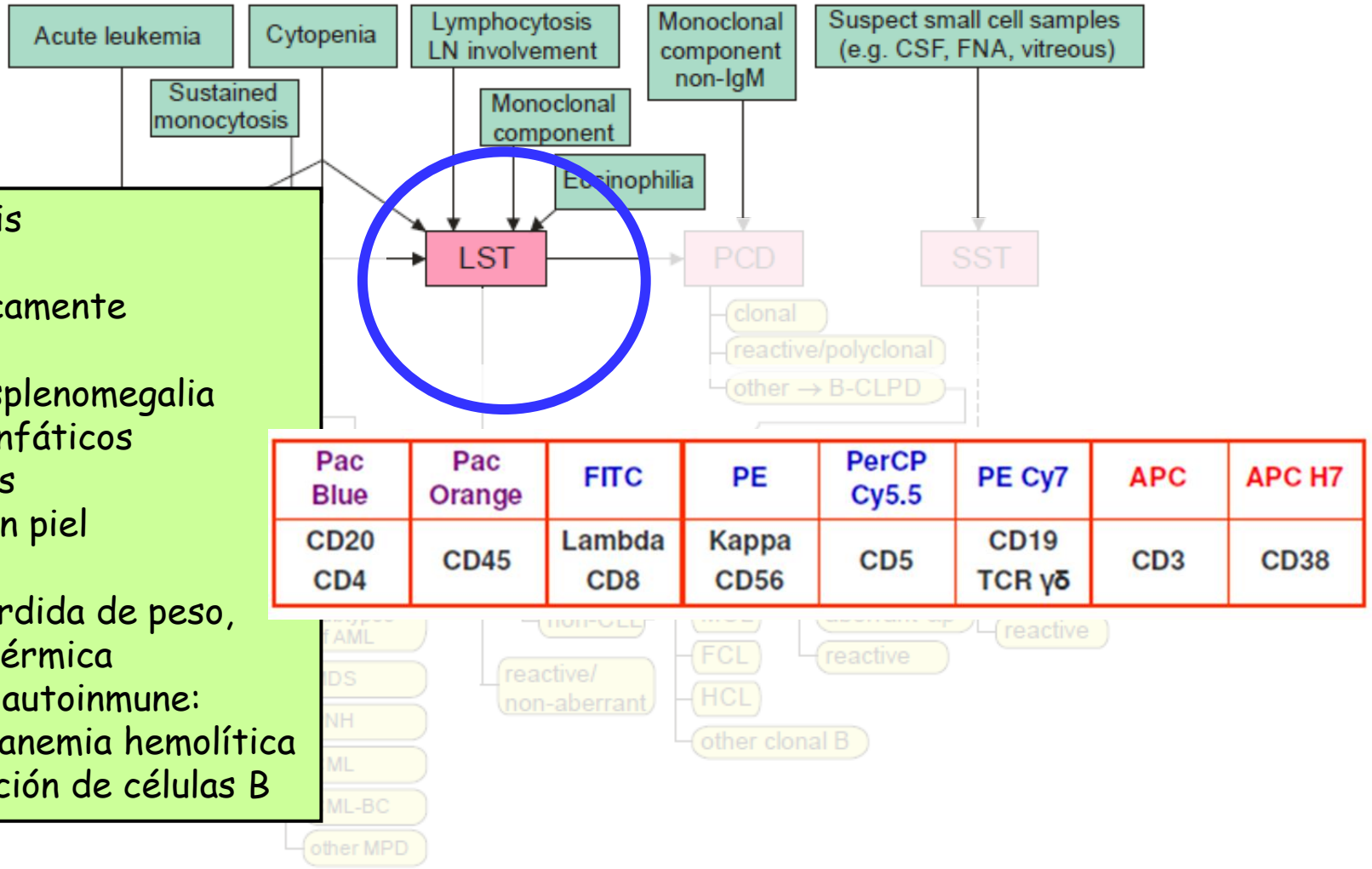


Inmunoreguladoras

NK CD56+
CD16++
90%

NK CD56++
CD16-/+
10%

Linfocitosis
Linfocitos morfológicamente anormales
Hepato/esplenomegalia
Ganglios linfáticos agrandados
Lesiones en piel
Eosinofilia
Fiebre, pérdida de peso, erupción dérmica
Fenómeno autoinmune: citopenia, anemia hemolítica
Desregulación de células B



Pac Blue	Pac Orange	FITC	PE	PerCP Cy5.5	PE Cy7	APC	APC H7
CD20 CD4	CD45	Lambda CD8	Kappa CD56	CD5	CD19 TCR γδ	CD3	CD38

Figure 1. Diagnostic flow diagram showing the potential usage of the EuroFlow antibody panels. Multiple entries are possible, which are dependent on the clinical-diagnostic question and on the availability of cell material. For example, analysis with the "small sample tube" (SST) can be followed by analysis with additional antibody panels (see dotted line), if sufficient cell material is left over.

Van Dongen et al, Leukemia 2012;26:1908-1975

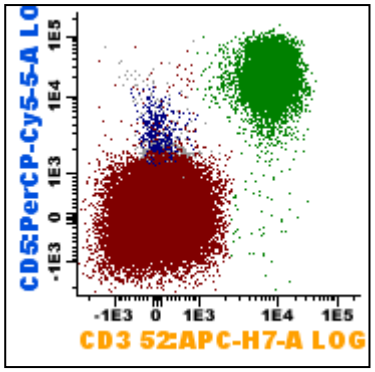
EuroFlow

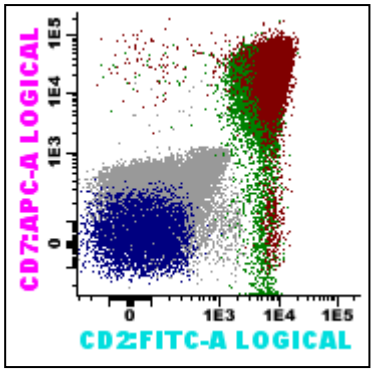
Panel para SLP-NK

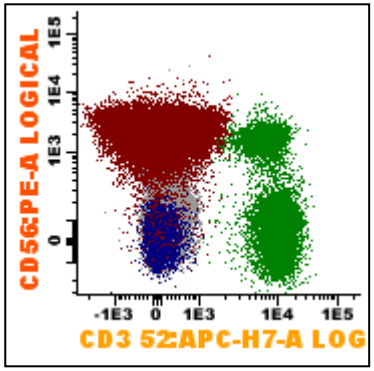
Tube	PacB	PacO	FITC	PE	PerCPCy5.5	PECy7	APC	APCH7
1	CD2	CD45	CD7	CD26	SmCD3	CD56	CD5	CD19
2	CD16	CD45	CD57	CD25	SmCD3	CD56	CD11c	CD19
3	HLADR	CD45	CyPerforin	CyGranzyme B	SmCD3	CD56	CD94	CD19

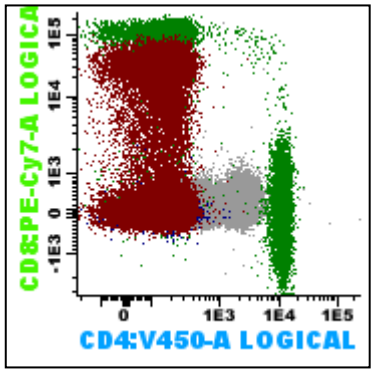
EuroFlow

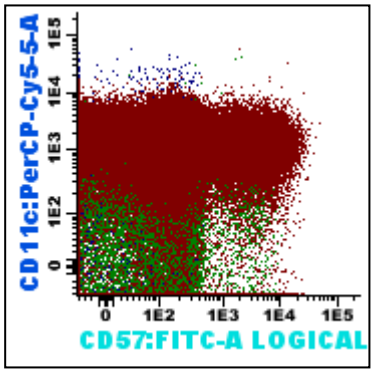
Leucemia LGL NK

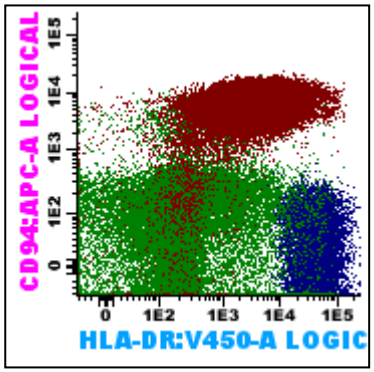


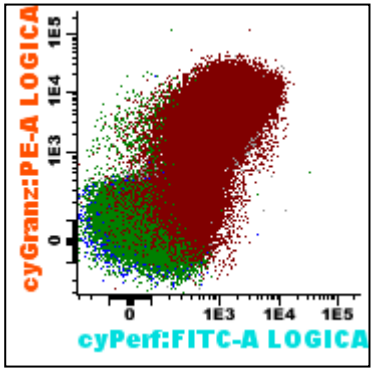


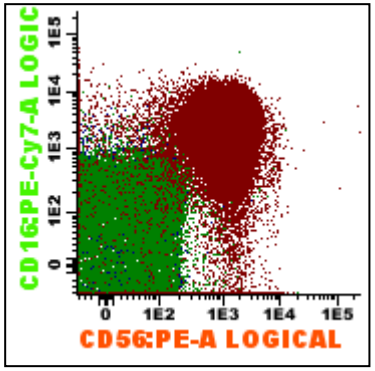


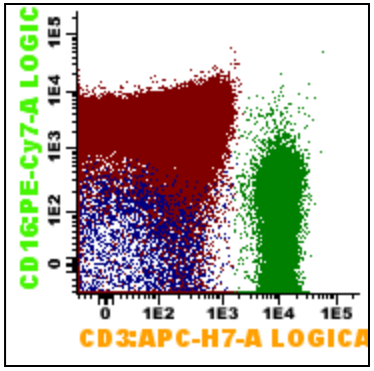


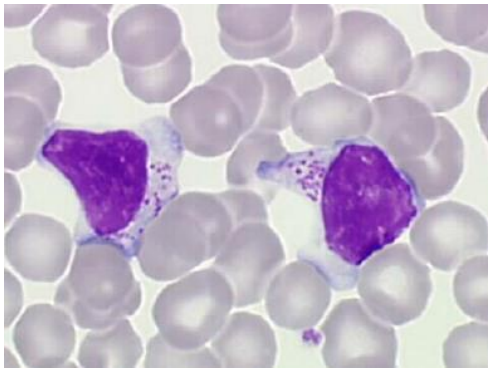






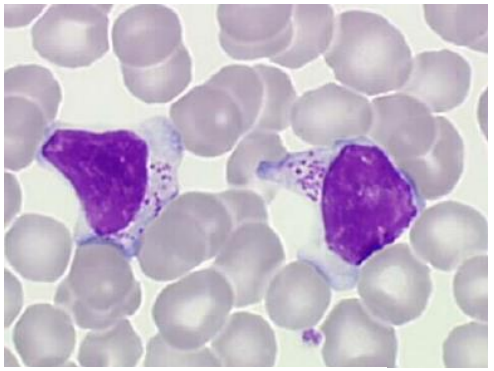






Afecta adultos y mas frecuente mujeres
Pacientes asintomáticos o con citopenias. No organomegalias
Curso estable o progresa lentamente
Asociada a neoplasias e infecciones virales HIV HCV

Leucemia LGL NK



NK-LGL leukemia cells are CD2+, CD5-, CD3 / TCRα/β–

Absence of CD26, CD27 and CD28 molecules is the role

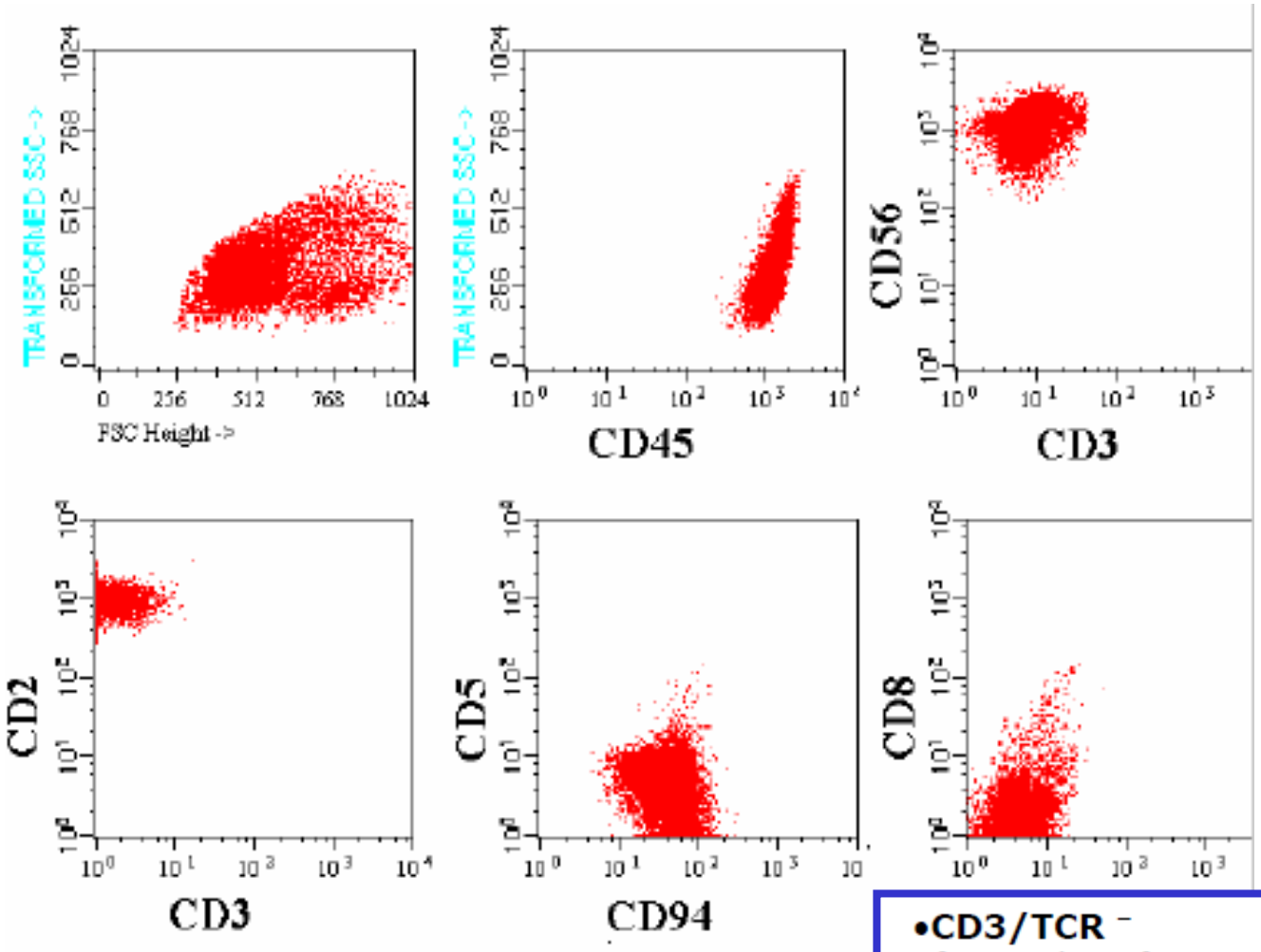
CD7 expression is usually dim and heterogeneous, whereas that of CD2 is usually strong homogeneous.

Absent / dim **CD56** and **CD11b** expression is typically observed, together with strong and homogeneous **CD11c** and **CD94** expression

Other **NK-cell associated markers** (CD16, CD57) and **Killer receptors** (CD158, CD161, etc) are variable.

Activation related markers (CD38 and HLA-Dr) are variably expressed in a variable number of cells; CD25 is always negative.

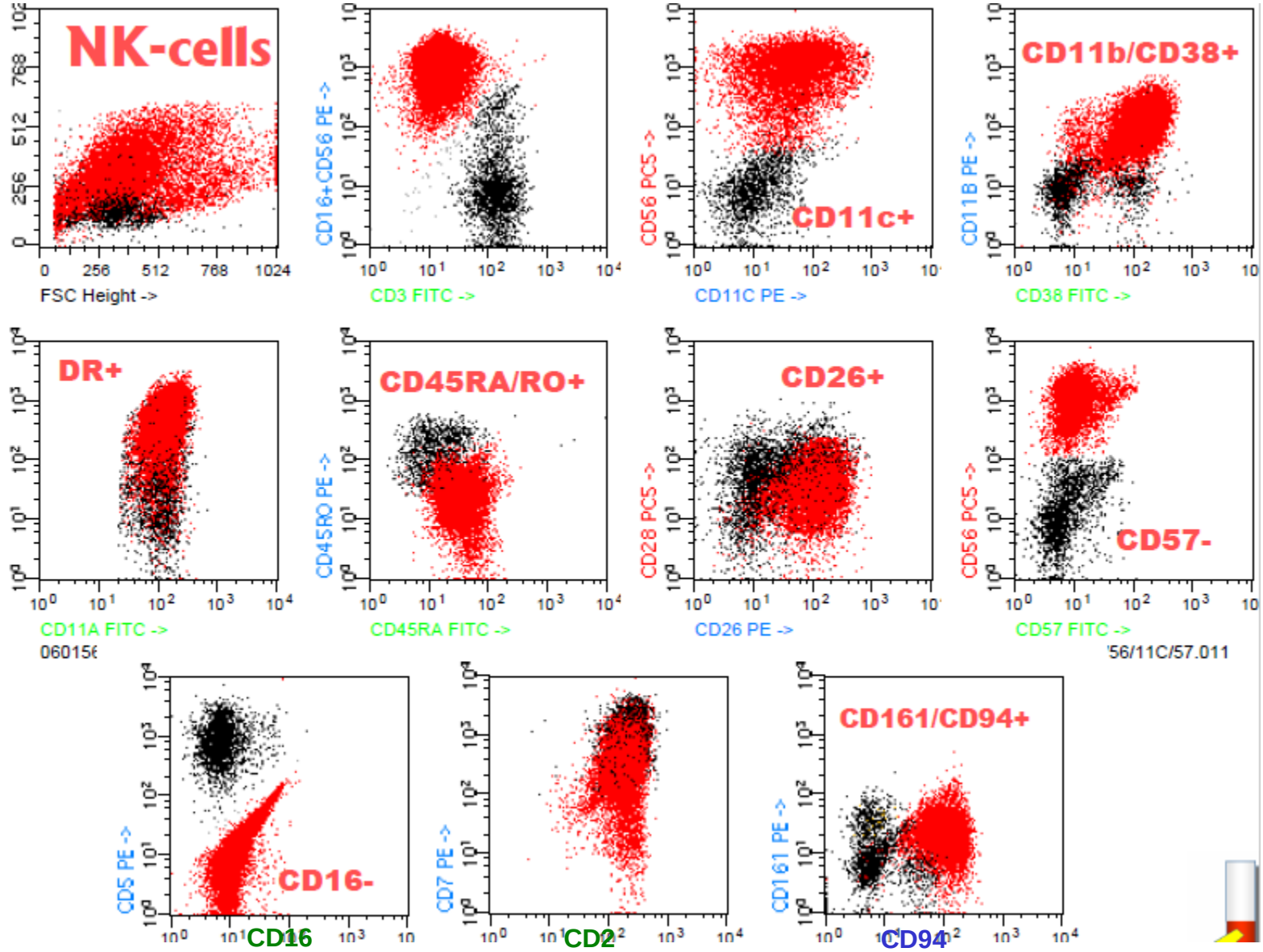
Linfoma de célula NK tipo nasal



- CD3/TCR $^{-}$
- (CD3ε $^{+}$ cyt)
- CD4 $^{-}$ CD8 $^{-/+}$
- NKa $^{+}$ (CD56^{+}, CD16$^{+/-}$, CD57 $^{-}$)
- Granzyme/perforine +

Linfoma extranodal agresivo
Compromete tracto resp superior, tracto GI o piel
EBV presente en 80-100%

Linfoma de célula NK tipo nasal



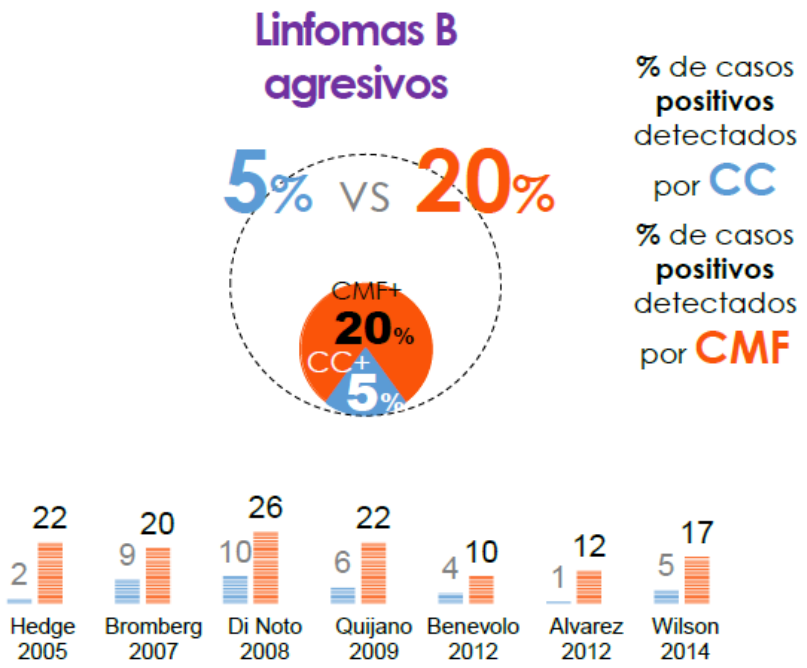
Neoplasias de células NK

Ausencia de criterios bien definidos para determinar clonalidad de células NK por inmunofenotipificación

No hay marcadores clonofenotípicos para células NK como ser las cadenas livianas de Igs κ y λ o el repertorio TCR Vβ en linf B y T respectivamente.
No hay una definición precisa de marcadores y perfiles clono-fenotípicos.

LCR

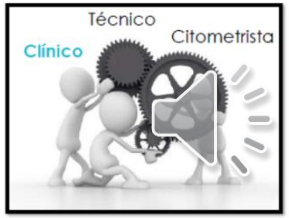
La CMF es **más sensible y específica** que la citología Convencional para la detección de células tumorales en LCR



Aspectos a tener en cuenta para el procesamiento de LCR por CMF

- ✓ BAJA CELULARIDAD
- ✓ BAJA CONCENTRACIÓN
- ✓ VOLUMEN LIMITADO DE MUESTRA
- ✓ CORTA VIABILIDAD CELULAR

- Evitar la pérdida de celular: solución estabilizante (Transfix)
- Evitar contaminación con sangre periférica
- Conocer fenotipo al diagnóstico



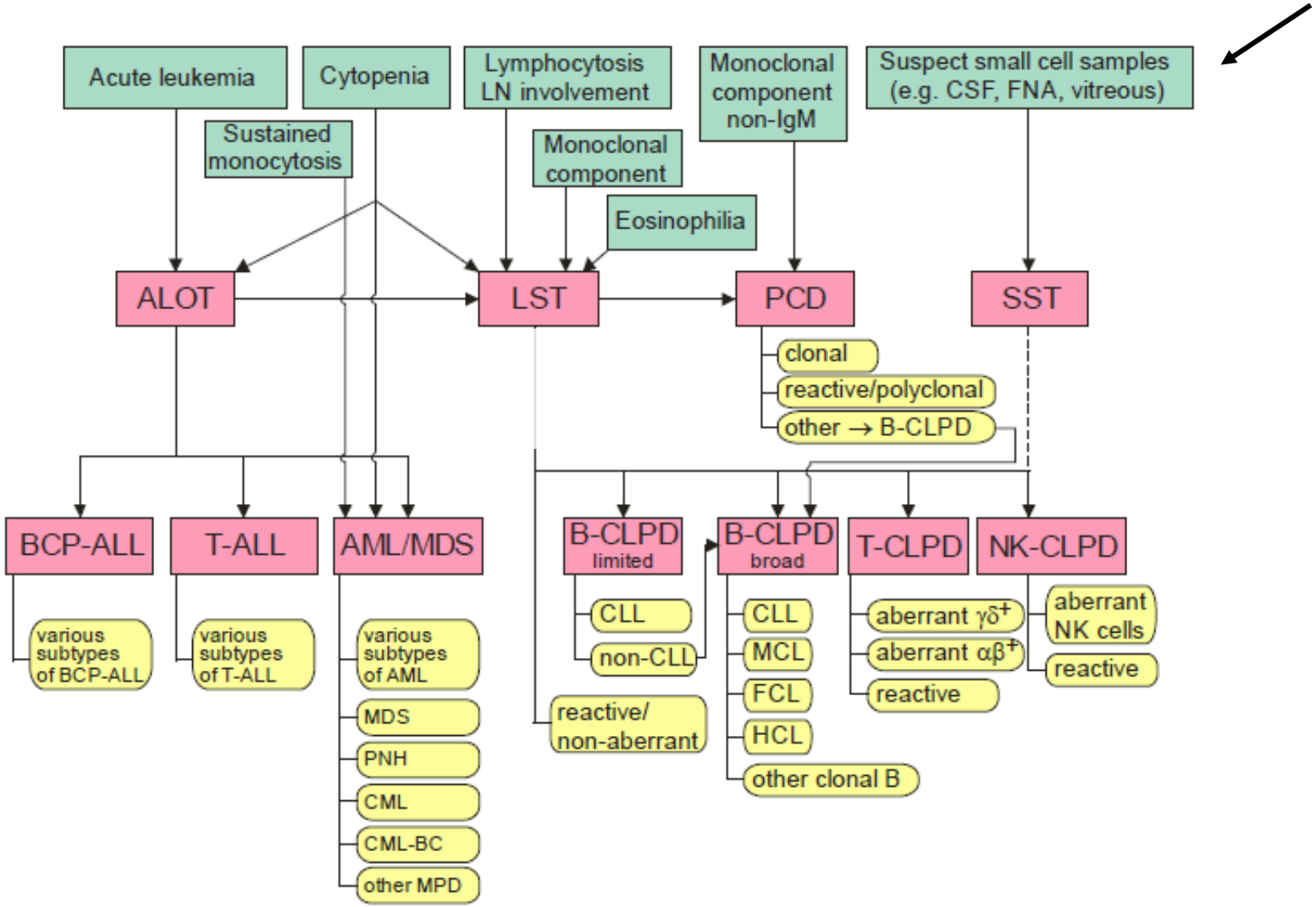
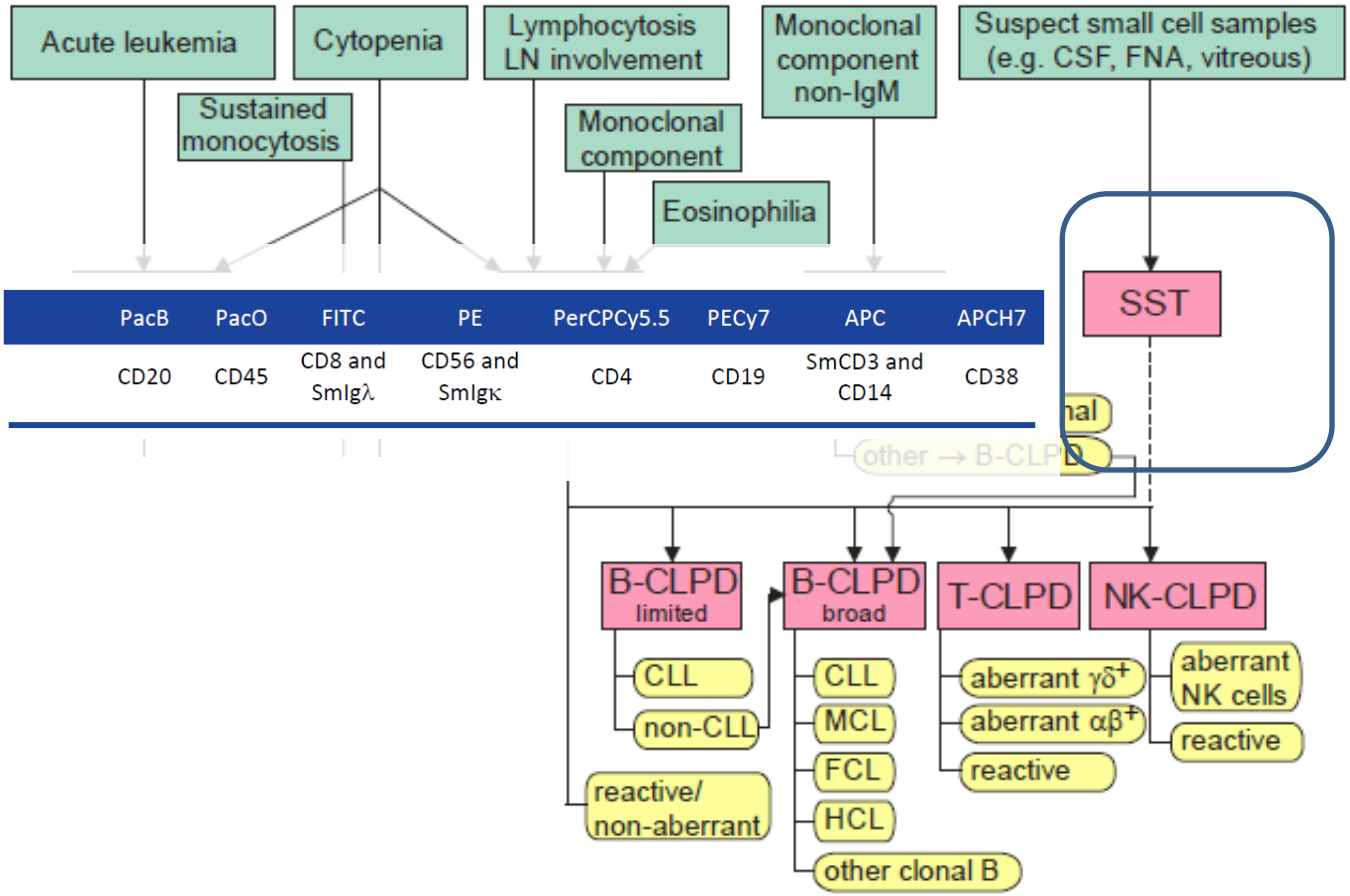


Figure 1. Diagnostic flow diagram showing the potential usage of the EuroFlow antibody panels. Multiple entries are possible, which are dependent on the clinical-diagnostic question and on the availability of cell material. For example, analysis with the "small sample tube" (SST) can be followed by analysis with additional antibody panels (see dotted line), if sufficient cell material is left over.

EuroFlow

Van Dongen et al, Leukemia 2012;26:1908-1975



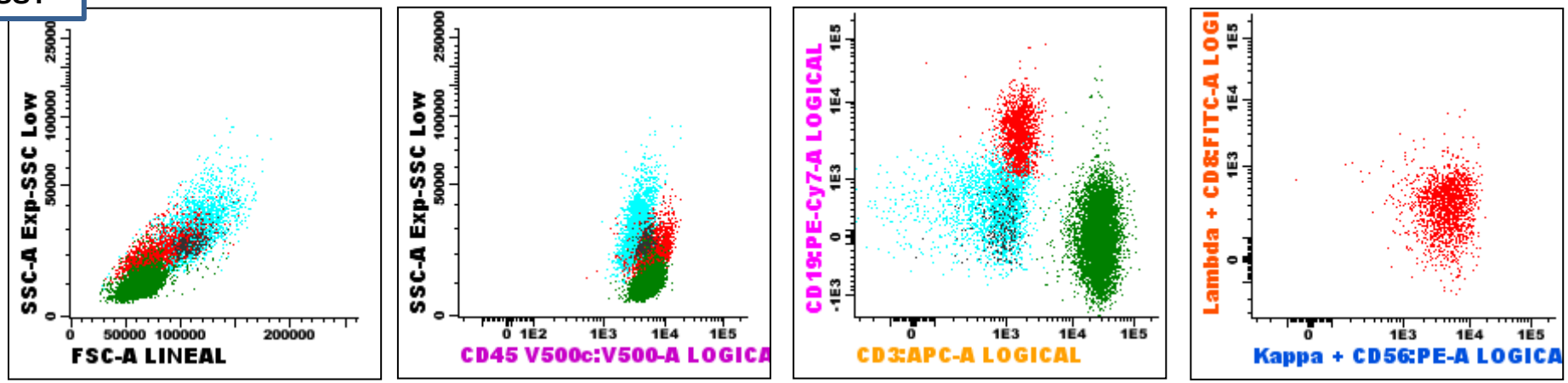
Van Dongen et al, Leukemia 2012;26:1908-1975

EuroFlow

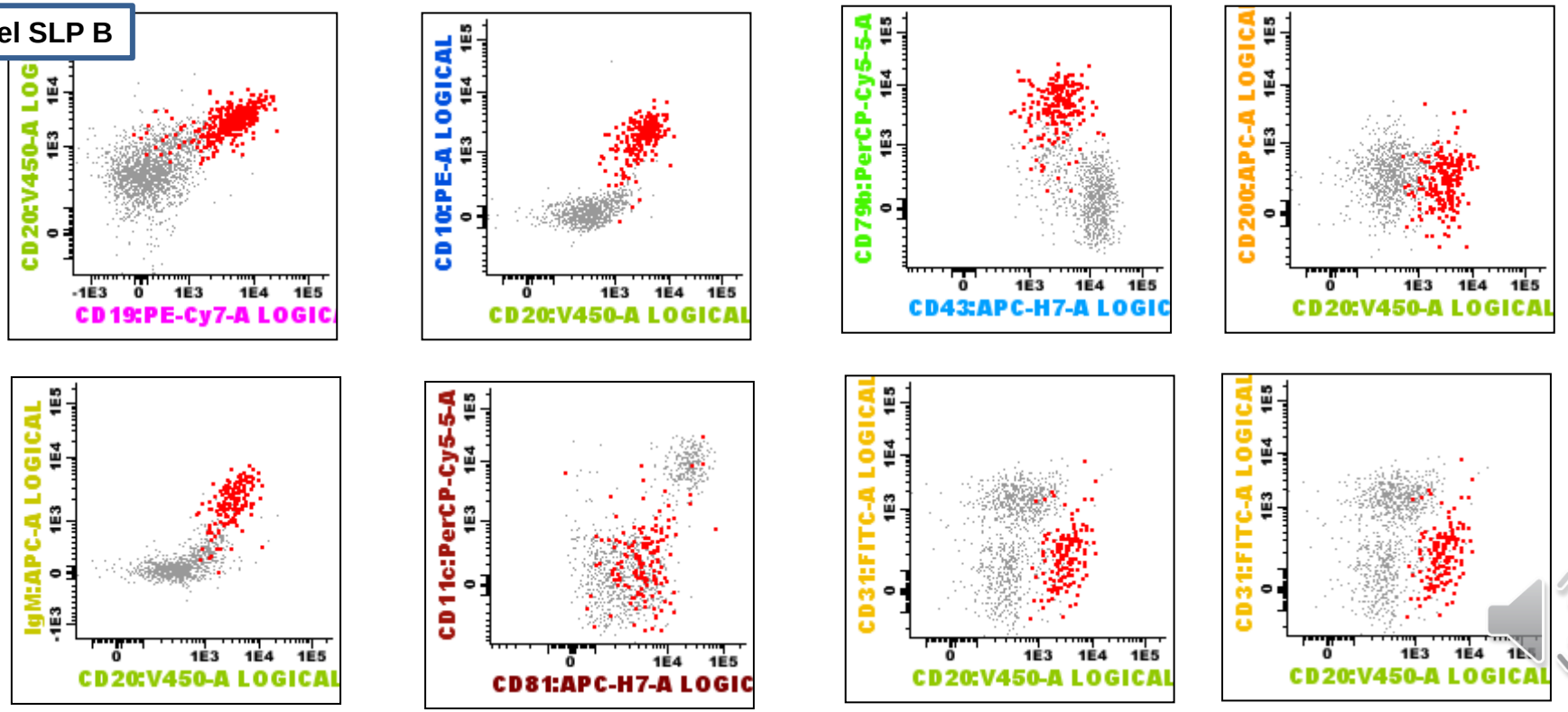
LCR

Linf. T Monocitos Linf. B patológicos

SST

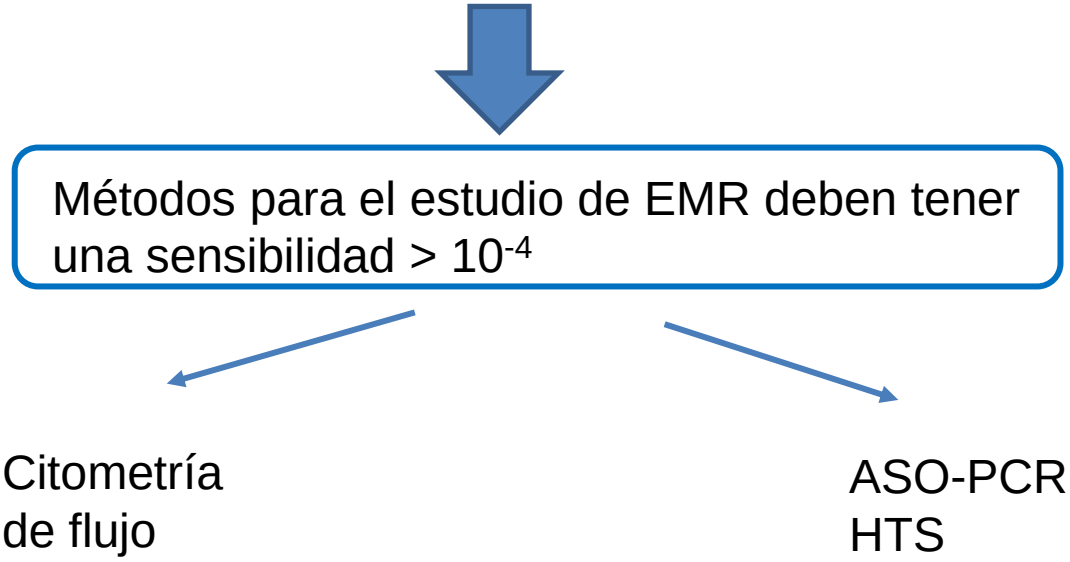


Panel SLP B



ENFERMEDAD MÍNIMA RESIDUAL EN LLC

Se considera **EMR negativa** cuando el paciente presenta menos de 1 célula leucémica en 10.000 leucocitos normales, en sangre periférica o médula ósea (sensibilidad del 0,01% o 10^{-4}).

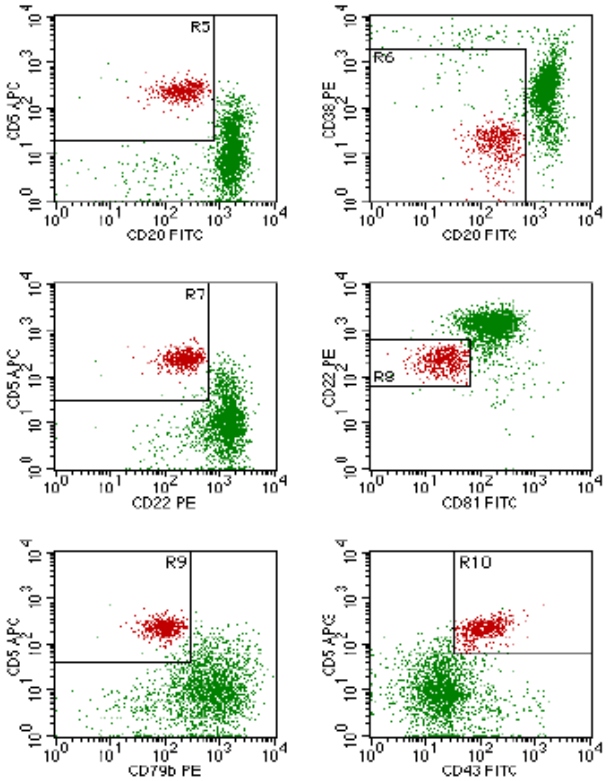


Flow Cytometry MRD Detection

Linf B normales
Linf B de LLC

Parameters	Tubes	Detection limit	Cells required for 0.01% – LoD
6 (4-colour)	4	0.005%	4–20 million
8 (6-colour)	2	0.001%	2–10 million
10 (8-colour)	1	0.001%	1–5 million

V450	V500	FITC	PE	PerCP-Cy5.5	PE-Cy7	APC	APC-H7
CD 5	CD 3	CD 81	CD 79b	CD 22	CD 19	CD 43	CD 20



Mary Sartor Sydney → 10-colour flow assay

Much better reagents became available

Requests to include: CD45, CD38, CD160, CD200, CD305, ROR1

LoD, limit of detection.

ERIC european research initiative on CLL

BD

Rawstron AC, *et al. Leukemia* 2016; **30**:929-936;
Rawstron AC, *et al. Leukemia* 2013; **27**:142–149;
Rawstron AC, *et al. Leukemia* 2007; **21**:956–964.

ENFERMEDAD MÍNIMA RESIDUAL EN LLC

- ✓ Es importante conocer el límite de detección y de cuantificación
  CFM: LoD 20 eventos, LoQ 50 eventos.
  Eventos totales > 2 millones para una sensibilidad de 10 -5

- ✓ Factores que impactan en los resultados
  CFM: fenotipo atípicos o cambios fenotípicos asociados al tratamientos

- ✓ Efecto compartimento: muestras hemodiluidas
  CFM: determinar poblaciones celulares minoritarias de la MO

Conclusiones

- Es necesario conocer la sospecha clínica para comenzar el estudio.
- La CFM es una técnica rápida, sensible para el diagnóstico, provee información extremadamente útil y completa en menos de 24hs.
- La CFM es una técnica complementaria y para llegar a un diagnóstico definitivo se debe correlacionar con la manifestación clínica y estudios complementarios.
- La propuesta Euroflow ha logrado la estandarización de esta compleja metodología para obtener resultados comparables entre laboratorios. Permite identificar/clasificar la mayoría de las entidades definidas por WHO.
- CFM es una técnica con altas sensibilidad para el monitoreo de EMR.

Gracias !!!

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Mail: belenvenegas78@hotmail.com

POSADAS
HOSPITAL NACIONAL

IAF
INSTITUTO
ALEXANDER
FLEMING